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INDUCTION OF TUMOURS IN MICE AND RATS WITH FERRIC SODIUM GLUCONATE AND IRON DEXTRAN GLYCEROL GLYCOSIDE

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THE carcinogenic properties of iron macromolecular complexes in rats and mice were described in 1959 and the early 1960's (Richmond, 1959; Haddow and Horning, 1960; Baker *et al.*, 1961; Lundin, 1961; Fielding, 1962). Since that time, a number of iron-containing compounds have been tested and found to induce subcutaneous tumours in various experimental animals (see Roe, 1967). In the course of a general survey of the carcinogenicity of iron-containing compounds, 2 further substances—ferric sodium gluconate and iron dextran glycerol glycoside—have emerged as agents with definite carcinogenic activity in rats and mice.

FERRIC SODIUM GLUCONATE COMPLEX

Materials and Methods

Forty male CB stock mice, aged 11 weeks, were used. The animals were housed in metal cages in groups of 5 and maintained on cubed diet No. 86 (Messrs. Dixon, Ware, Herts.) and water *ad libitum*.

Ferric sodium gluconate complex was obtained from Dr. Kutiak and Co., Arzneimittelfabrik, Vienna. It was supplied in 2 ml. ampoules, each of which contained 150 mg. iron. Tests were carried out on Batch No. 22 01 11.

Twenty animals received 17 weekly subcutaneous injections of ferric sodium gluconate in the right flank—0·1 ml. for the first 3 weeks and 0·05 ml. for the following 14 weeks. The total amount of iron injected was 75 mg. Twenty untreated mice served as controls.

The animals were examined daily. Sick mice were killed and the survivors were killed 16 to 18 months after the beginning of the experiment. Complete post-mortem examinations were carried out and tissues showing macroscopic abnormalities were fixed in Bouin's solution. Paraffin sections 5 μ thick were prepared and stained with haematoxylin and eosin.

RESULTS

The survival of mice in the test and control groups, together with the incidence of local and distant tumours, is shown in Table I. Injection-site tumours developed in 5 test animals—the first after 10 months and the last after 15 months. Once palpable, they grew rapidly and it was necessary to kill the mice within 30 days of the first appearance of a definite subcutaneous mass. The morphology of these neoplasms was similar to that reported previously in animals injected with iron-preparations. All of them were spindle cell or pleomorphic sarcomas,

showing variable degrees of differentiation. A few iron-containing macrophages were present in and around the tumours but no iron-pigment was seen in the tumour cells themselves. No metastases were found. Two of the sarcomas were successfully transplanted into other mice of the same stock strain.

The injection sites in mice which did not develop local tumours showed the usual changes associated with prolonged parenteral administration of iron. The flanks were thickened, indurated, and hairless. The subcutaneous tissues were stained brown and contained large numbers of macrophages laden with iron. Multinucleate giant cells were sometimes seen, together with a few chronic inflammatory cells. Fibrous tissue was increased in all animals.

The number and distribution of distant neoplasms in the test mice were low (Table I). Malignant lumphomas were found in 2 animals, one of which also developed an injection-site sarcoma.

TABLE I.—*Induction of Tumours in Mice by Ferric Sodium Gluconate*

	Age (months)					
	3	6	9	12	15	18
Test animals						
Survivors	20	20	19	18	14	0
Tumours (cumulative totals)						
Injection-site	0	0	0	2	4	5
Other	0	0	0	0	0	2*
Control animals						
Survivors	20	11	8	5	3	0
Tumours	0	0	0	1*	2*	5*

* All malignant lymphomas

Various non-malignant changes were commonly encountered in other tissues. Increased amounts of iron-pigment were seen in macrophages in the axillary and inguinal lymph nodes, spleen and pancreas and in hepatic Küpffer cells. Fatty change and necrosis were sometimes observed in hepatocytes but this was not apparently related to the amount of iron present in the liver. Slight atrophy of pancreatic acinar cells. bronchiectasis, and bronchopneumonia were also seen in some animals.

Five untreated mice from the control group developed malignant lymphomas. No other tumours were seen and the incidence of non-malignant changes such as hepatic degeneration and pulmonary infection was similar to that found among the test animals.

IRON DEXTRAN GLYCEROL GLYCOSIDE

Materials and Methods

One hundred and five male CB stock mice were divided into 3 test groups and 1 untreated control group. The animals were aged 11 weeks and maintained as in the previous experiment. In addition, 48 male CB stock rats were used. These animals were 8 weeks old and were housed in metal cages, 4 in each: they were fed cubed diet No. 86 and water *ad libitum*.

Iron dextran glycerol glycoside was obtained from Dr. P. G. Marshall. The Nicholas Research Institute, Slough, Bucks. It was supplied in ampoules containing 50 mg. iron/ml. Tests were carried out on Batch numbers A 2533 and O 3214.

The test animals received subcutaneous injections of iron dextran glycerol glycoside into the right flank according to the scheme shown in Table II:

TABLE II.—*Treatment of Mice and Rats with Iron Dextran Glycerol Glycoside*

No. of animals	No. of injections	Dose per injection (ml.)	Total amount of iron administered (mg.)
Mice			
Group 1 20	5, weekly	0·1	25
Group 2 25	8, weekly	0·2	80
Group 3 20	29, fortnightly	0·05	75
Group 4 40	Uninjected controls		—
Rats			
Group 1 24	25, weekly	0·5	625
Group 2 24	Uninjected controls		—

The subsequent care of the animals, the post-mortem examinations, and the selection and staining of tissues for histological examination were as described previously.

Results

Effects in mice.—Although 52 mice in the 3 test groups lived for more than 12 months after the beginning of the experiment, only one developed a sarcoma at the site of injection, a tumour which appeared after 11 months in an animal from Group 2. The injection sites in the remaining 104 mice showed the usual changes associated with repeated subcutaneous injections of iron compounds.

The incidence of distant tumours was high in both test and control groups. Malignant lymphomas, including thymomas, were the commonest lesions, followed by hepatomas and pulmonary adenomas. One mouse from Group 1 developed a squamous carcinoma of the forestomach with metastasis to the omental fat, mesentery and diaphragm.

Non-neoplastic changes in distant tissues consisted of deposits of iron-pigment in macrophages in the liver, spleen, pancreas and occasionally the kidneys of mice injected with the iron compound. Test and control animals showed fatty change and patchy necrosis of hepatic parenchyma and pulmonary infection.

Effects in rats.—The survival of test and control rats, together with the incidence of injection-site sarcomas, is recorded in Table III. Among the test

TABLE III.—*Induction of Tumours in Rats with Iron Dextran Glycerol Glycoside*

	Age (months)								
Test animals	3	6	9	12	15	18	21	24	27
Survivors	24	24	24	22	18	10	5	2	0
Tumours (cumulative totals)									
Injection-site	0	0	0	0	2	4	8	10	12
Other	0	0	0	0	1[a]	3[b, c]	3	3	3
Control animals									
Survivors	24	24	22	21	11	8	5	4	0
Tumours (cumulative totals)	0	0	0	0	1[d]	2[e]	3[f]	3	3

[a] = mammary carcinoma
[b] = mammary fibroadenoma
[c] = solitary exocrine adenoma of pancreas
[d] = hepatoma
[e] = malignant lymphoma
[f] = subcutaneous fibroma

animals, 12 developed local tumours, the first after 13 months and the last after 25 months. They grew rapidly and the animals were killed 20 to 30 days after the lesions were first observed. Of the 12 neoplasms seen, 10 were pleomorphic or spindle cell sarcomas, similar in histological appearance to those which developed in mice injected with ferric sodium gluconate. There were also 2 fibromas. No metastases were observed.

The incidence of distant neoplasms among the test animals was low (Table III). Of the 3 tumours found, only one—a solitary exocrine adenoma of the pancreas—was seen in an animal which already had an injection site sarcoma. The non-malignant changes in distant tissues were similar to those described in mice injected with iron dextran glycerol glycoside except that there was more morphological evidence of accumulations of iron in tissues such as the spleen, liver and pancreas.

Three tumours were found among the untreated control rats—a mammary fibroadenoma, a mammary carcinoma and a subcutaneous fibroma from the occipital region.

DISCUSSION

It is clear that repeated subcutaneous injections of ferric sodium gluconate induce local sarcomas in mice and that iron dextran glycerol glycoside, administered in a similar fashion, induces injection-site tumours in rats. In both instances, the animals received doses of iron which were large in relation to their body weight but the part played by iron-overloading (cf. Golberg *et al.*, 1960) in tumour induction by these 2 compounds cannot be assessed. The difficulty is emphasised by the observation that while ferric sodium gluconate induced a number of injection-site sarcomas in mice, iron dextran glycerol glycoside (even in large and prolonged doses) showed negligible carcinogenic activity in the same species. Another feature is the apparent difference in carcinogenic potency of iron dextran glycerol glycoside in rats and mice. Although the total amount of iron administered to the mice was higher, on a body weight basis, than that given to the rats, the carcinogenic response was strikingly less. In previous investigations on macromolecular iron complexes, the response of the 2 species has usually been broadly similar.

Since different dose-schedules were used in the 2 experiments, it is not possible to compare the sarcomas induced in mice with ferric sodium gluconate, and in rats with iron dextran glycerol glycoside, in terms of their final incidence and times of induction. Histologically, however, the sarcomas were similar in the 2 groups and resembled the tumours induced by other iron-containing compounds; such lesions have frequently been described and illustrated in previous papers. One difference between rats and mice which emerged from the present study was the tendency for rats—but not mice—to develop injection-site fibromas. Fibromas are not uncommon in rats injected with macromolecular iron complexes (e.g. Roe *et al.*, 1964; Roe and Carter, 1967) but we have not seen such tumours in mice, nor are they described in other accounts dealing with the carcinogenicity of iron-compounds in mice. If this apparent species difference is a valid one, it suggests that the final neoplastic response of the subcutaneous tissues to repeated injections of iron-containing substances may be significantly different in rats and mice. Differences between rats and mice in terms of the amount of iron retained

at injection sites have been reported (Golberg *et al.*, 1960; Baker *et al.*, 1960) but differences in the type of tumour produced have not been noted previously.

It is still uncertain whether iron-containing compounds are likely to induce an increase in the incidence and variety of neoplasms in tissues distant from the site of injection (Roe and Carter, 1967). But in the present study, the incidence of distant tumours in mice treated with ferric sodium gluconate, and in rats treated with iron dextran glycerol glycoside, was unusually low. Distant tumours were more numerous in mice injected with iron dextran glycerol glycoside but, as emphasised earlier, a high incidence of neoplasms was also found in the corresponding group of untreated control animals. One of the tumours encountered in a test mouse—the locally-metastasising squamous carcinoma of the forestomach—is certainly a rarity (Rowlatt, 1967) but its relationship to treatment with iron dextran glycerol glycoside is obscure.

The present findings provide more information on the carcinogenicity of iron-containing compounds in rats and mice but they do nothing towards resolving the controversy concerning the carcinogenic hazards of such compounds in man (Haddow and Horning, 1960; Baker *et al.*, 1961; Haddow *et al.*, 1964; Roe, 1966). As Haddow and his colleagues have stressed, it is still doubtful whether parenteral iron preparations have been used in clinical practice for a sufficient period of time to be certain that such materials are not carcinogenic. The therapeutic value of iron-containing compounds is beyond dispute but, at the present time, it seems reasonable to urge caution in the selection of patients and duration of treatment and to avoid the indiscriminate use of such substances.

SUMMARY

Five out of 20 mice which received 17 once-weekly subcutaneous injections of ferric sodium gluconate (total 1 ml.) developed spindle cell or pleomorphic sarcomas at the injection site.

Ten out of 24 rats which received 25 once-weekly injections of 0·5 ml. of another proprietary preparation—iron dextran glycerol glycoside—also developed local sarcomas; in addition, 2 developed local fibromas. Of 104 mice given 5 injections of 0·1 ml., 8 injections of 0·2 ml. or 29 injections of 0·05 ml. of the same preparation, only 1 developed a neoplasm at the site of injection.

Differences between mice and rats in their response to injected iron compounds are discussed and the apparent rarity of local fibromas in mice is emphasised.

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Pharmacological Research on Sodium Gluconate

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I

Introduced in therapy in the last fifteen years, calcium gluconate soon found favor with the physicians, so much so that its indications now extend to a great many diseases. But it is especially in pulmonary tuberculosis that the drug is used, usually by intravenous injection of concentrated solutions (5-10 cc of 10% solutions each time). As the use of the remedy spread, shortcomings have been pointed out by many authors, for which as a rule no plausible reason could be found, especially since the chemical analysis of the preparation always showed it to be perfectly pure.

It has been said of calcium gluconate that it is about four times less toxic than calcium chloride (Rothlin, 1), and this affirmation, subsequently assumed by all to be correct, is still being used by the therapists to proclaim it to be the ideal remedy in therapy. However, research done at the Institute by Baldacci (2) showed, by comparative experiments rigorously carried out by the method of Simon (3), that calcium gluconate is not four times less toxic than the chloride but only one and a half times. Baldacci further showed that at equal dose the drug produces a more profound and durable lowering of the temperature than does the chloride. This profound perturbation of the temperature may perhaps explain the drawbacks found by many authors (hypothermia, cardiac insufficiency, more or less severe lipothymia, etc.)

As happens all too often is therapy today, the drugs are used on the patient without extensive preparatory studies on laboratory animals to explore their action from every point of view. This has been the case with calcium gluconate. We have therefore carried out at this Institute much research on

the drug in question and are still doing so in order to obtain a complete picture of its action to bridge the many gaps in our knowledge of its pharmacological aspects. To be able to study from all sides the pharmacological action of calcium gluconate it is necessary, of course, not only to make a comparison with the activity exerted by calcium chloride, the latter being an anion salt indifferent to the organism, but also to determine exactly the action of the gluconic anion. The only way to do this is to study the pharmacological action of sodium gluconate, which has a cation indifferent to the organism, thereby evidencing the effects of the anion. This has been the aim of the investigations described in this paper.

II Experiments

Sodium gluconate has the formula $CH_2OH.(CHOH)_4.COONa$ and its molecular weight is 218.088. I used the high-purity salt prepared by Fraenkel & Laudau of Berlin. The experiments consist of several series. In a first series I studied the general action exerted by the drug on the higher animal (rabbit) and determined its remote minimum lethal dose by intravenous application according to Simon (3). In a second test series I investigated the action of the drug on the isolated toad heart. In a third series I undertook to study how sodium gluconate acts on the circulation of the hind section of toads prepared according to Lawen-Trendelenburg. In a last series I recorded the arterial pressure and respiration of rabbits which had been given rapid intravenous injections of solutions of the salt to the death of the animal.

First Test Series - General action of sodium gluconate and its remote minimum lethal dose in the rabbit.

I prepared two solutions of the salt in distilled water - one 0.5 n (109 g in 1000 cc) and one normal solution (218 g in 1000 cc). Having selected rabbits in a good state of health and having strapped them stretched out on a retaining table, I started to inject the 0.5 n solution in the marginal vein

of an ear. I soon saw that the dose of 40 cc per kg was very well tolerated. For this reason I adopted the use of the normal solution. The rate of injection was in all tests 0.5 cc per kg of body weight and per minute. In all animals treated I always noted the breathing frequency per minute, the frequency of the heart beats per minute, and the rectal temperature, both before starting to apply the drug and during the entire experiment. I also observed the symptoms which the animal exhibited during the entire test, during the injection of the solution as well as thereafter, that is, until the animal either regained a nearly normal condition or succumbed. In the latter case I performed an autopsy and removed the organs (lungs, liver, spleen, kidneys) for the histological examination which I then carried out with the usual technique (fixation in Zenker's solution, inclusion in paraffin, staining with hematoxylin and eosin).

Rather than reporting the detailed description of the individual experiments, I shall limit myself to summarizing the basic data thereof in Table I and to stating succinctly the symptomatology presented by the rabbits under the drug being studied.

It is seen from the table that the remote minimum lethal dose by intravenous application in the rabbit (that is, the smallest dose which, injected in the veins at a rate innocuous in itself, kills the animal after a certain interval from the injection) is 7.630 g (or 0.035 g-eq.) of sodium gluconate per kg of body weight.

As to the symptomatology observed, it should be distinguished in two different types according as the animal survives or dies. In the first case, when the dose is very small (exp. 1), the animal shows no particular symptoms; during the entire injection it is lively, the heart beats are not changed as to rhythm, number or intensity; the breathing is negligibly reduced; the only noteworthy fact is the lowering of the temperature by two and a half

Table I

Test No.	Sodium gluconate solution 0.5 n (cc per kg)	Sodium gluconate solution n (cc per kg)	Duration of injection	Sodium gluconate g per kg	Sodium gluconate g - eq. per kg	Outcome
I	30	-	60 min	3.270	0.015	Lives
II	40	-	80	4,360	0.020	Lives
III	-	30	60 min	6,540	0.030	Lives
VI	-	33	66 min	7,194	0.033	Lives
VII	-	35	70 min	7,630	0.035	Dies after ca 24 h
V	-	36	72 min	7,848	0.036	Dies after ca 21 h
IV	-	40	80 min	8,720	0.040	Dies after ca 15 h

degrees at the end of the injection. At higher, but not lethal doses, the lowering of the temperature persists and may be as much as 3 degrees. The heart beats remain the same in their characteristics, while changes variable from animal to animal are noted in the breathing. For example, a considerable increase appears in the second experiment in the number of respirations per minute, which increase is maintained as long as the injection lasts and then abates, while in the third experiment there appears immediately a rather noticeable reduction, and in the sixth experiment a slight initial increase in the frequency of respiration precedes a subsequent decrease. This different behavior may have something to do with the state the animal was in when the experiment was started. For example, in the third experiment, before the injection was started the rabbit was breathing fast and the frequency of respiration decreased rapidly as soon as injecting was started.

Other symptoms observed in these animals treated with non-lethal doses of the drug were diffuse tremors or slight muscular contractions, which ceased when the injection was stopped. At the highest dose (7.194 g/kg, sixth exper-

iment) also an increasing weakness of the animal was found, which prevented it from standing up on its legs when the injection was finished, but which then gradually disappeared.

In the animals which perished (seventh, fifth, fourth experiments) no grave disorders occurred in the cardiac activity; the temperature sometimes dropped by as much as 5.8°C (fourth experiment); in the number of respirations an increase was nearly always found, which was never very great (fourth and seventh experiment), but sometimes there was no increase (fifth experiment). The animals sometimes showed muscular contractions and especially an increasing dejection, to the point that they did not support themselves on their limbs when untied from the retaining apparatus. These conditions grew worse - respiration and heart weakened more and more, and death occurred 15 to 24 hours after the end of the injection. The autopsy and histological examination showed only a slight congestion in all organs, without any other noteworthy fact. The death must therefore probably be attributed to the depressive action of the drug on the central nervous system.

Second Test Series - Action of sodium gluconate on isolated toad heart

For these experiments I used big toads weighing about 80 grams. Having isolated the animal's heart, I introduced therein a Straub cannula. The sodium gluconate solutions used had been prepared in Ringer's solution for lower animals. Their concentrations varied from 2.18 g of the salt in 10 million cc of Ringer (= 0.000,001 g-mol per liter) to 2.18 g in 10 cc (= 1.0 g-mol per liter).

The graph shown in figure 1 gives a clear picture of the action exerted by the salt on the isolated heart.

It is seen that the more dilute solutions L, I, H, G (whose concentration varies from 2.18 g in 10 million to 2,18 in 10,000) do not appreciably alter the activity of the heart either as to amplitude or as to number per unit time. Of the more concentrated solutions, F (2.18 g in 1000) proves to have a slight exciting effect on the activity of the organ (increase in amplitude; negligible

increase in frequency). Solution E (2.18 g in 100) produces a very noticeable reduction of the strokes, while the frequency is not altered. Subsequent washing with Ringer's solution completely restores the activity of the organ. Lastly, solution D (2.18 g in 10) stops the heart, and only after prolonged repeated washing with Ringer's solution do the cardiac movements become apparent, but in a quite rudimentary manner.

To recapitulate, only with the solution 2.18:1000 was it possible to evidence a weakly exciting action of sodium gluconate on the isolated toad heart. The more dilute solutions were indifferent for the activity of the organ. One must go to a concentration of 2.18:100 to obtain a clear depressive action.

Fig. 1 Action of sodium gluconate on the isolated toad heart.

R, Ringer

L, Solution of sodium gluconate 2.18 : 10 million = 0.000,001 g-mol per ltr

I, " " " " 1 " 0.000,01 "

H, " " " " 100,000 0.000,1 "

G, " " " " 10,000 0.001 "

F, " " " " 1,000 0.01 "

E, " " " " 100 0.1 "

D, " " " " 10 1.0 "

Time signal every 5 sec.

Third Test Series - Action of sodium gluconate on the circulation of the hind section of toads.

For these experiments I took big toads. Having destroyed the animal's medulla and brain, I prepared the circulation in the hind section according to Lawen-Trendelenburg, following the known rules. I let Ringer's solution flow through, and after some time I let pass the solutions of sodium gluconate and Ringer alternately, for 6 minutes. I counted the drops that flowed from the

specimen during the fifth and sixth minute that the tested liquid was passing. The averages of the number of drops flowing per minute are shown in Table II, which sets forth the results obtained in a very demonstrative experiment of this series.

Table II

Circulation in toad hind section. Preparation acc. to Lawen-Trendelenburg

Solution used	Concentration g Na gluconate in cc of Ringer	g-mol of Na glue per liter	Number of drops in 60 sec	Remarks
Ringer	-	-	22	
Na gluconate	2.18:10,000,000	0.000,001	26	
Ringer	-	-	28	
Na gluconate	2.18:1,000,000	0.000,010	32	
Ringer	-	-	33	
Na gluconate	2.18:100,000	0.000,100	38	
Ringer	-	-	39	
Na gluconate	2.18:10,000	0.001,000	39	
Ringer	-	-	41	
Na gluconate	2.18:1,000	0.010,000	44	
Ringer	-	-	42	
Na gluconate	2.18:100	0.100,000	51	Myofibril contractions diffused over entire body
Ringer	-	-	44	
Na gluconate	2.18:10	1.000,000	24	(The soln.2.18:10 is rather dense, syrupy, quite viscous)
Ringer	-	-	25	

It can be seen from Table II that the very dilute solutions of sodium gluconate in Ringer (2.18 g in 10 million) have a vasodilatating action. It increases with increasing concentration of the solution and reaches a maximum at the concentration 2.18:100. With further increase of the concentration of the solution a vaso-constricting action occurs which brings the caliber of the vessel back almost to the starting point. (As to the reduction in the number of drops flowing from the specimen, we must, of course, not neglect the influence also of the considerable density of the solution at concentration 2.18;10). Washing with Ringer does not subsequently change the caliber of the vessels.

Fourth Test Series - Action of sodium gluconate on arterial pressure and on respiration in the rabbit

The technique adopted in this test series is the following. An animal in perfect physiological condition was secured on the retaining apparatus. Having isolated the left carotid, I introduced in its cardiac stump a cannula communicating with a mercury manometer equipped with a stylus writing on a rotating smoked cylinder. At the same time I introduced into the trachea a T-shaped cannula, one branch of which communicated with the outside while the other was connected by a rubber tube with a Marey drum equipped with a stylus writing on the same smoked cylinder. Lastly, having isolated the right jugular vein, I introduced in its central stump a glass cannula which communicated by a rubber tube with a graduated test tube containing a normal solution (218 g in 1000 cc) of sodium gluconate. I now report two of the experiments made.

Experiment I - Rabbit weighing 1.620 kg. The injection of the normal solution of sodium gluconate was conducted at the rate of 2 cc per kg of body weight and per minute. The experiment is illustrated in figure 2.

Examination of the graph shows that as soon as the sodium gluconate is introduced, a decrease in the amplitude of respiration and in the frequency

is obtained in a few seconds, while the pressure shows a lowering similar to that obtained by stimulation of the vagus at the neck. The pressure then rises again until the normal level is reached, while breathing becomes a little deeper but less frequent. Suddenly the pressure drops while respiration becomes rare, incomplete and shallower by degree until a period of apnea is reached. While the pressure continues to decrease, the breathing takes on a periodical rhythm (six groups separated by pauses) until it stops. After breathing has stopped, the heart continues pulsating for a certain time.

Fig. 2 Action of sodium gluconate on arterial pressure and respiration in the rabbit (Experiment I).

First sign: Start of injection into the right jugular vein of a normal solution of sodium gluconate at the rate of 2 cc per kg of body weight and per minute.

Second sign: End of injection of the solution. A total of 19.61 cc of solution per kg have been injected, corresponding to 4.2749 g, or 0.0196 g-eq. of sodium gluconate per kg.

Time signal every 5 sec.

Experiment II - Rabbit weighing 1.650 kg. The injection of the normal solution of sodium gluconate is conducted at first at the rate of 1 cc per kg and per minute. Then the rate of injection of the drug is doubled. The test results obtained are shown in figure 3 (graphs 1 and 2).

It is evident at once that as soon as the injection of the sodium gluconate solution starts (Graph 1), an increase in amplitude and frequency of respiration appears, which increases with increasing dose of the drug. It is further seen in this graph that after a slight increase the pressure becomes normal again, but then it undergoes a slight but progressive decrease while amplitude and frequency of respiration increase considerably. In the second

graph, at a certain moment corresponding to the doubling of the rate of injection of the drug, the amplitude of respiration still increases while the frequency decreases considerably, and the arterial pressure continues its slight and progressive decline. Later the respiratory movements diminish in frequency and amplitude and the pressure continues to go down, slightly at first, sharply later. Finally respiration stops and the pressure continues to decrease tending toward zero more rapidly. But when the respiration stops, the heart still continues to beat for a while, more and more weakly.

It follows from the experiments just reported that the respiratory activity is modified to some extent by the various doses of the drug: Being at first excited by the slow rates of injection until a maximum is reached, this activity then progressively diminishes and finally breathing stops altogether. The arterial pressure, after a brief initial increase, also demonstrable only for the slow rates of injection, begins to decrease and this decrease is magnified when respiration is about to stop so that the pressure goes down rapidly when respiration ceases. The heart still continues to beat when respiration has stopped entirely.

Fig. 3 (Graphs 1 and 2) - Action of sodium gluconate on arterial pressure and on respiration in the rabbit (Experiment II)

Graph 1, first sign: Start of injection of a normal solution of sodium gluconate in the right jugular vein at the rate of 1 cc per kg and per minute.

Graph 2, first sign: The rate of injection is doubled.

second sign: Injection of the drug is stopped. In all there have been injected 40.30 cc of solution per kg, corresponding to 8.7854 g, or 0.0403 g-eq. of sodium gluconate per kg.

Time signal every 5 sec.

In conclusion, these experiments confirm what we have seen before in connection with the study of the general action of the drug on the rabbit. We had observed that the cardiac activity is little changed, but that there were clearer modifications in respiratory function. These experiments, therefore confirm the former. The greatest and clearest changes are seen in the respiration. When there already appears a considerable increase in the respiratory activity, the increase in pressure is negligible, and while the changes in amplitude and frequency of respiration become outstanding, the arterial pressure exhibits a slight, progressive reduction quite different from the conspicuous changes in respiration. Only when the respiratory activity undergoes a rather sharp decline, the arterial pressure suddenly drops markedly and approaches zero. But when breathing has ceased, the cardiac activity goes on and finally cease a few minutes later.

III Conclusions

It can be affirmed on the basis of research in the pharmacology of sodium gluconate that:

1. The remote minimum lethal dose by intravenous application of sodium gluconate is, in the rabbit, 7.630 g (= 0.035 g-eq.) per kg of body weight;

2. The death of the higher animal (rabbit) occurs with a progressive weakening of the forces by the depressive action exerted by the drug on the central nervous system;

3. While the solutions of the drug at low concentration (from 2.18 g in 10 million to 2.18 g in 10,000) are indifferent on the activity of the isolated toad heart, an exciting action is demonstrable only for rather concentrated solutions (2.18 g in 1000). Much more concentrated solutions stop it altogether;

4. T he action of sodium gluconate on the isolated vessels of the hind section of toad (preparation according to Lawen-Trendelenburg) is clearly dilating, and only at very high doses the drug tends to bring the vessel caliber

back to a condition fairly similar to the physiological;

5. The respiratory activity of the rabbit is generally heightened by the intravenous administration of sodium gluconate. At suitable injection rates a marked excitation of the respiration is evidenced, which can increase the activity of the respiratory apparatus tremendously. This period of intense excitation is followed by a depressive period which leads to the paralysis of the respiratory function;

6. The blood pressure is at first slightly increased by moderate doses of the drug, then follows a prorgressive reduction which tends to bring the pressure to zero. When breathing tends to stop, the pressure drops suddenly, and when breathing ceases, it undergoes a further reduction, while the heart still continues to beat for a few minutes.

Summary

The author shows that in the rabbit the remote minimum lethal dose of sodium gluconate by intravenous application, is 7.630 g (=0.035 g-eq.) per kg of body weight and that the death of the animal occurs with a progressive reduction of the forces due to the depressive action exerted by the drug on the central nervous system. On the isolated heart of toads sodium gluconate has a weak exciting action only in fairly concentrated solutions, while very concentrated solutions stop it altogether. On the isolated vessels of the hind section of toad the drug always shows a conspicuously dilating action, except for very high concentrations, which tend to bring the vessel caliber back to the initial values. On the respiration the drug has a definitely exciting action at suitable doses; higher doses exert a depressive action on the respiration and finally stop it altogether. The blood pressure goes up slightly at first at moderate doses of the drug only; it then decreases continuously approaching zero. The cardiac activity continues for a few minutes after breathing has stopped.

(Translated by Carl Demrick Associates, Inc/LH/t)

sodium glue. 3

ARCHIVIO DI FARMACOLOGIA SPERIMENTALE

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RICERCHE FARMACOLOGICHE SUL GLUCONATO DI SODIO

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I.

Introdotto in terapia nell'ultimo quindicennio, il gluconato di calcio ha ben presto incontrato il più largo favore tra i medici, tanto che le sue indicazioni si sono estese alla massima parte delle forme morbose. Ma è specialmente nella tubercolosi polmonare che il farmaco viene impiegato per lo più a mezzo di iniezioni endovenose di soluzioni concentrate (cc. 5-10 di soluzione al 10 % per volta). Coll'estendersi dell'uso del rimedio non ne sono mancati inconvenienti, segnalati da molti Autori, e per i quali di regola non si seppe trovare ragione plausibile, dato specialmente che l'analisi chimica del preparato dimostrò in ogni caso la sua perfetta purezza.

Del gluconato di calcio si disse che è circa quattro volte meno tossico del cloruro di calcio (ROTHLIN) (1) e di questa affermazione, ammessa poi da tutti come esatta, i terapisti si valsero e si valgono per proclamarlo il rimedio ideale in terapia. Però ricerche praticate in Istituto da BALDACCI (2) dimostrarono, con esperienze comparative rigorose condotte a mezzo del metodo di SIMON (3), che il gluconato di calcio non è quattro volte meno tossico del cloruro ma solamente una volta e mezza. Il BALDACCI inoltre dimostrava che il farmaco produce, a parità di dosi equivalenti, una diminuzione della temperatura assai più profonda e duratura di quanto non faccia il cloruro. Questo profondo perturbamento della temperatura può forse spiegare gli inconvenienti lamentati da molti

Autori (ipotermie, insufficienze cardiache, lipotimie più o meno gravi, ecc.).

Come oggi troppo spesso avviene in terapia, i farmaci vengono impiegati sul malato senza studi preparatori vasti sugli animali di laboratorio che ne sviscerino da ogni punto di vista l'azione. Così è accaduto per il gluconato di calcio. Perciò in questo Istituto furono fatte molte ricerche sul farmaco in questione e molte se ne fanno ancora in modo da poter ottenere un quadro completo della sua azione che colmi le molte lacune esistenti nelle conoscenze delle sue attitudini farmacologiche. Ma è chiaro che, affinchè sia possibile studiare da ogni lato l'azione farmacologica del gluconato di calcio, non soltanto è necessario ricorrere al confronto con l'attività esplicata dal cloruro di calcio, essendo quest'ultimo un sale ad anione indifferente per l'organismo, ma è anche necessario conoscere esattamente l'azione dell'anione gluconico. Ciò non può ottenersi se non studiando l'azione farmacologica del gluconato di sodio che ha un catione indifferente per l'organismo, il che permette di mettere in evidenza gli effetti dell'anione. A questo fine sono volte le indagini che formano l'argomento del presente lavoro.

II. — ESPERIENZE

Il gluconato di sodio ha la formula $CH_2OH.(CHOH)_4.COONa$ ed ha p. m. uguale a 218,088. Ho adoperato il sale purissimo preparato dalla casa Fraenkel e Landau di Berlino. Le esperienze constano di diverse serie. In una prima serie studiai l'azione generale esplicata dal farmaco sull'animale superiore (coniglio) e ne determinai la dose minima letale lontana per via endovenosa secondo Simon (3). In una seconda serie di esperienze indagai l'azione del farmaco sul cuore isolato di rospo. In una terza serie mi proposi di studiare il modo col quale il gluconato di sodio agisce sul circolo del treno posteriore di rospo preparato alla Läwen-Trendelenburg. In un'ultima serie, infine, mi interessai della registrazione dei tracciati della pressione arteriosa e del respiro di conigli in cui praticavo rapide iniezioni endovenose di soluzioni del sale fino alla morte dell'animale.

1ª Serie di esperienze. — *Azione generale del gluconato di sodio e sua dose minima letale lontana nel coniglio.*

Preparai due soluzioni del sale in acqua distillata: una soluzione 0,5 N (g. 109 in cc. 1000) ed una soluzione N (g. 218 in cc. 1000). Scelti i conigli in buone condizioni di salute, fissatili proni ad un tavolo di contenzione, cominciai ad iniettare nella vena marginale di un orecchio la soluzione 0,5 N. Vidi ben presto che la dose di cc. 10 per Kg. era tollerata benissimo. Per questo motivo passai senz'altro all'impiego della soluzione N. La velocità dell'iniezione in tutte le esperienze fu di cc. 0,5 per Kg. di peso corporeo e per minuto primo. In tutti gli animali trattati ho sempre annotata la frequenza degli atti respiratori per minuto, la frequenza delle pulsazioni cardiache per minuto e la temperatura rettale. E ciò tanto prima d'iniziare l'inoculazione del farmaco che durante tutto lo svolgimento dell'esperienza. Ho pure osservata la sintomatologia che l'animale presentava durante tutto l'esperimento, sia durante l'iniezione della soluzione che successivamente a questa, vale a dire sino a che l'animale si fosse rimesso in condizioni pressochè normali oppure venisse a morte. In questo caso ne praticavo l'autopsia e raccoglievo gli organi (polmoni, fegato, milza, reni) per l'esame istologico che poi eseguivo con la usuale tecnica (fissaggio in Zenker, inclusione in paraffina, colorazione con ematossilina ed eosina).

Anziché riportare la descrizione particolareggiata delle singole esperienze, per maggiore brevità mi limito a riassumerne i dati fondamentali nella tabella I e ad esporre in sintesi la sintomatologia presentata dei conigli per effetto del farmaco in istudio.

TABELLA I.

Numero della esperienza	Soluzione di gluconato di sodio		Durata della iniezione	Gluconato di sodio		Esito
	0,5 N (cc. per Kg.)	N (cc. per Kg.)		g per Kg.	g - eq. per Kg.	
I	30	—	60′	3,270	0,015	Vive
II	40	—	80	4,360	0,020	Vive
III	—	30	60′	6,540	0,030	Vive
VI	—	33	66′	7,194	0,033	Vive
VII	—	35	70′	7,630	0,035	Muore dopo circa 24 ore
V	—	36	72′	7,848	0,036	Muore dopo circa 21 ore
IV	—	40	80′	8,720	0,040	Muore dopo circa 15 ore

Dall'esame della tabella risulta che la dose minima letale lontana per via endovenosa nel coniglio (cioè la dose più piccola che iniettata nelle vene con una velocità per sè stessa innocua uccide l'animale ad una certa distanza dall'iniezione) è di g. 7,630 (corrispondenti a g.-eq. 0,035) di gluconato di sodio per Kg. di peso corporeo.

Per quanto riguarda la sintomatologia osservata, conviene distinguerla in due tipi diversi a seconda che l'animale sopravvive alla dose di farmaco ricevuta oppure muore. Nel primo caso, quando la dose è molto piccola (esperienza 1) l'animale non mostra sintomi particolari: durante tutta l'iniezione è vivace, i battiti cardiaci non si modificano nè per ritmo, nè per numero, nè per intensità; gli atti respiratori presentano una trascurabile diminuzione; unico fatto degno di nota è l'abbassamento della temperatura di due gradi e mezzo alla fine dell'iniezione. Con dosi più forti, ma non letali, persiste l'abbassamento della temperatura, abbassamento che può arrivare a 3 gradi. I battiti cardiaci non si modificano mai nei loro caratteri, mentre nel respiro si rilevano variazioni diverse da animale ad animale. Per esempio, nella seconda esperienza appare un aumento notevole nel numero degli atti respiratori al minuto, aumento che permane finchè si fa l'iniezione e che si va attenuando poi, mentre nell'esperienza terza appare subito una diminuzione assai ragguardevole e nell'esperienza sesta si ha un lieve aumento iniziale della frequenza del respiro che precede una diminuzione successiva. E' probabile che tale contegno diverso del respiro possa essere messo in rapporto con lo stato in cui si trova l'animale quando si incominciò l'esperienza. Ad esempio, nella esperienza terza il coniglio, prima che si cominciasse l'iniezione, aveva un respiro frequente che diminuì rapidamente della metà la sua frequenza appena si cominciò ad iniettare.

Altri sintomi osservati in questi animali trattati con dosi non letali del farmaco furono tremori diffusi oppure lievi contrazioni muscolari che cessarono quando si sospese l'iniezione. Con la dose più elevata (g. 7,194 per Kg., esperienza sesta) fu rilevata anche una debolezza crescente dell'animale che gli impediva di mantenersi ritto sulle zampe ad iniezione finita, ma che poi sparì a mano a mano che ci si allontanava dal termine dell'iniezione.

Negli animali venuti a morte (esperienze settima, quinta, quarta) non si ebbero durante l'iniezione disturbi gravi dell'attività cardiaca; la temperatura diminuì talora anche di 5°,8 C. (esperienza quarta); nel numero degli atti respiratori si rilevò quasi sempre un aumento che non fu mai fortissimo (esperienze quarta e settima), ma talora l'aumento mancò (esperienza quinta). Gli animali mostrarono talora contrazioni muscolari e soprattutto un abbattimento crescente al punto che, slegati dall'apparecchio di contenzione, non si reggevano sugli arti. Queste condizioni si andarono aggravando: respiro e cuore si indebolirono progressivamente e la morte intervenne in periodi varianti da 15 a 21 ore dalla fine dell'iniezione. L'autopsia e l'esame istologico non mostrarono che una leggera congestione in tutti gli organi, senza alcun altro fatto degno di nota. La morte deve perciò probabilmente attribuirsi all'attività depressiva esercitata dal farmaco sul sistema nervoso centrale.

2ª Serie di esperienze. — *Azione del gluconato di sodio sul cuore isolato di rospo.*

Per queste esperienze ho adoperato grossi rospi del peso di circa 80 grammi. Isolato il cuore dell'animale, vi introducevo una cannula *Straub*. Le soluzioni di gluconato di sodio impiegate erano fatte in *Ringer* per animali inferiori. Le loro concentrazioni variavano da g. 2,18 del sale in 10 milioni di cc. di *Ringer* (= g.-mol. 0,000.001 per litro) a g. 2,18 in cc. 10 (=g.-mol. 1,0 per litro).

Il tracciato riportato nella figura 1 dà una chiara visione dell'azione esercitata dal sale sul cuore isolato.

Risulta dal tracciato che le soluzioni più diluite L, I, H, G (la cui concentrazione varia da g. 2,18:10 milioni a g. 2,18:10.000) non modificano in modo apprezzabile l'attività del cuore sia per quanto riguarda l'ampiezza delle escursioni che per quanto si riferisce al loro numero nell'unità di tempo. Delle soluzioni più concentrate, la F (g. 2,18:1000) si dimostra dotata di lieve effetto eccitante sull'attività dell'organo (aumento dell'ampiezza; trascurabile aumento della frequenza). La soluzione E (g. 2,18 : 100) produce una notevolissima diminuzione delle escursioni cardiache, mentre la frequenza non può dirsi modificata. Il lavaggio successivo con *Ringer* ripristina completamente l'attività dell'organo. Infine, la soluzione D (g. 2,18 : 10) arresta il cuore e solamente dopo prolungati e ripetuti lavaggi con *Ringer* i movimenti cardiaci si rendono palesi ma in maniera affatto rudimentale.

Riepilogando, solamente con la soluzione 2,18 : 1000 fu possibile mettere in evidenza un'azione debolmente eccitatrice del gluconato di sodio sul cuore isolato di rospo. Le soluzioni più diluite furono indifferenti per l'attività dell'organo. Bisognò toccare la concentrazione 2,18 : 100 per ottenere una netta azione depressiva.

3ª Serie di esperienze. — *Azione del gluconato di sodio sul circolo del treno posteriore di rospo.*

Per queste esperienze mi servii di grossi rospi. Distrutto il midollo ed il cervello dell'animale, preparai la circolazione nel treno posteriore alla Läwen-Trendelenburg, secondo le regole note. Vi facevo defluire il *Ringer* e dopo un certo tempo vi facevo passare le soluzioni di gluconato di sodio ed il *Ringer* alternativamente, per 6 minuti. Contavo le goccie che defluivano dal preparato durante il 5° ed

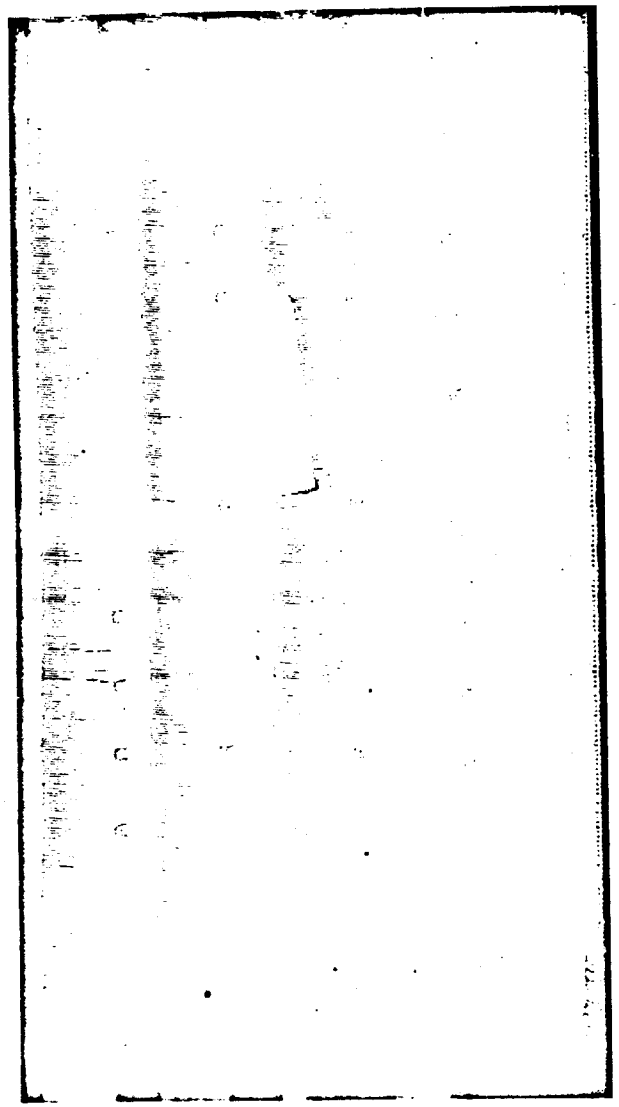

Fig. 1.

Azione del gluconato di sodio sul cuore isolato di rospo.

R, Ringer
L, Soluzione di gluconato di sodio 2,18 : 10 milioni = g-mol. 0,000.001 per L.
I, » » 2,18 : 1 milione = g-mol. 0,000.01 per L.
H, » » 2,18 : 100.000 = g-mol. 0,000.1 per L.
G, » » 2,18 : 10.000 = g-mol. 0,001 per L.
F, » » 2,18 : 1.000 = g-mol. 0,01 per L.
E, » » 2,18 : 100 = g-mol. 0,1 per L.
D, » » 2,18 : 10 = g-mol. 1,0 per L.

Segnale del tempo ogni 5".

il 6° minuto in cui passava il liquido in esame. I valori medi del numero delle gocce defluite per minuto sono riportati nella tabella II nella quale sono esposti i risultati ottenuti in una esperienza molto dimostrativa di questa serie.

TABELLA II.

Circolazione nel treno posteriore di rospo.

Preparazione alla LÄWEN - TRENDELENBURG.

Soluzione usata	Concentrazione		Numero delle gocce in 60"	Osservazioni
	g di glucon. di Na in cc di Ringer	g-mol. di gluc. di Na per litro		
Ringer	—	—	22	
Glucon. di Na	2,18: 10.000.000	0,000.001	26	
Ringer	—	—	28	
Glucon. di Na	2,18: 1.000.000	0,000.010	32	
Ringer	—	—	33	
Glucon. di Na	2,18: 100.000	0,000.100	38	
Ringer	—	—	39	
Glucon. di Na	2,18: 10.000	0,001.000	39	
Ringer	—	—	41	
Glucon. di Na	2,18: 1.000	0,010.000	44	
Ringer	—	—	42	
Glucon. di Na	2,18: 100	0,100.000	51	Contrazioni miofibrillari diffuse a tutto il corpo.
Ringer	—	—	44	
Glucon. di Na	2,18: 10	1.000.000	24	(La soluz. g. 2,18 : 10 è piuttosto densa, sciropposa, notevolmente viscosa)
Ringer	—	—	25	

Dall'esame della tabella II si può rilevare che le soluzioni molto diluite di gluconato di sodio in *Ringer* (g. 2,18 : 10 milioni) hanno azione vasodilatatrice. Questa, col crescere della concentrazione della soluzione, cresce alla sua volta fino a toccare il massimo colla soluzione g. 2,18 : 100. Si ha poi coll'aumento ulteriore della concentrazione

della soluzione un'azione vasocostrittrice la quale riporta il calibro vasale quasi al punto di partenza. (Per la diminuzione del numero delle gocce defluite dal preparato non va, naturalmente, trascurata anche l'influenza della notevole densità della soluzione a concentrazione 2,18 : 10). Il lavaggio col *Ringer* non modifica ulteriormente il calibro dei vasi.

4ª Serie di esperienze. — *Azione del gluconato di sodio sulla pressione arteriosa e sul respiro del coniglio.*

La tecnica adottata in questa serie di esperienze è la seguente. Scelto l'animale in condizioni perfettamente fisiologiche, lo fissavo sull'apparecchio di contenzione. Isolata la carotide sinistra, introduco nel suo moncone cardiaco una cannula comunicante con un manometro a mercurio munito di penna scrivente su cilindro ruotante affumicato. Introducevo nello stesso tempo in trachea una cannula a T, una branca della quale era in comunicazione coll'ambiente esterno, mentre l'altra era connessa mediante un tubo di gomma con un tamburo *Marey* munito di penna scrivente a sua volta sullo stesso cilindro affumicato. Infine, isolata la vena giugulare destra, introducevo nel suo moncone centrale una cannula di vetro che, mediante un tubo di gomma, comunicava con una provetta graduata contenente una soluzione N (g. 218 in cc. 1000) di gluconato di sodio. Riporto ora due delle esperienze fatte.

Esperienza I. — Coniglio di Kg. 1,620. L'iniezione della soluzione N di gluconato di sodio venne condotta alla velocità di cc. 2 per Kg. di peso corporeo e per minuto. L'esperienza è illustrata nella figura 2.

L'esame del tracciato dimostra che appena si comincia l'introduzione del gluconato di sodio si ottiene in pochi secondi una diminuzione dell'ampiezza del respiro ed anche della sua frequenza, mentre la pressione segna un abbassamento analogo a quello che si ottiene per stimolazione del vago al collo. La pressione poi risale fino a toccare il livello normale, mentre il respiro si fa un po' più ampio ma molto meno frequente. D'improvviso la pressione cade mentre gli atti respiratori si fanno rari, incompleti e s'impiccoliscono a grado a grado fino ad ottenersi un periodo di apnea. Mentre la pressione continua ad abbassarsi il respiro assume un ritmo periodico (sei gruppi separati da pause) finchè si arresta. Il cuore, a respiro arrestato, continua a pulsare per un certo tempo.

Esperienza II. — Coniglio di Kg. 1,650. L'iniezione della soluzione N di gluconato di sodio viene in un primo tempo condotta alla velo-

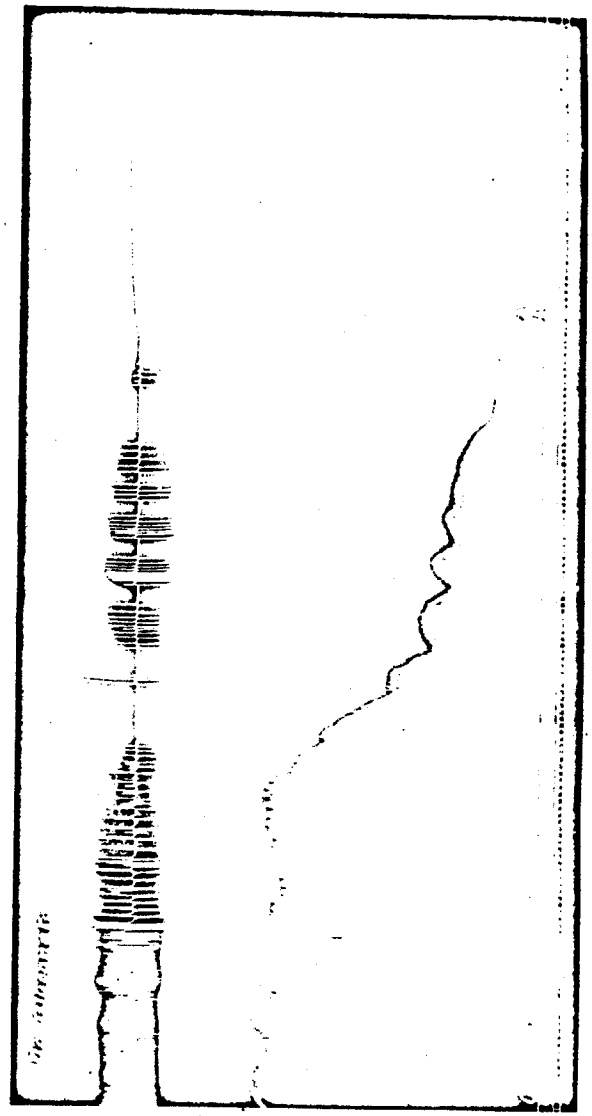

Fig. 2.

Azione del gluconato di sodio sulla pressione arteriosa e sul respiro del coniglio (Esperienza I).
1° segno: si incomincia ad inoculare nella vena giugulare destra una soluzione N di gluconato di sodio alla velocità di cc 2 per Kg. di peso corporeo e per minuto.
2° segno: termina l'iniezione della soluzione. Complessivamente sono stati inoculati cc 19,61 di soluzione per Kg. corrispondenti a g. 4,2749, cioè a g-eq. 0,0196 di gluconato di sodio per Kg.
Segnale del tempo ogni 5".

cità di cc. 1 per Kg. e per minuto. In un secondo tempo, si raddoppia la velocità d'inoculazione del farmaco. I risultati sperimentali ottenuti sono rappresentati nella figura 3 (tracciati 1° e 2°).

Il fatto che appare qui subito chiaro è che appena s'incomincia l'iniezione della soluzione di gluconato di sodio (Tracciato 1°) si rende palese un aumento dell'ampiezza e della frequenza degli atti respiratori, aumento che diventa sempre maggiore col crescere della dose del farmaco. In questo tracciato si vede, inoltre, che la pressione dopo un leggero aumento ridiventa normale, ma poi, mentre il respiro accresce notevolmente ampiezza e frequenza, essa subisce un abbassamento leggero ma progressivo. Nel secondo tracciato, ad un certo momento, corrispondentemente al raddoppiamento della velocità d'inoculazione del farmaco, l'ampiezza del respiro aumenta ancora mentre la frequenza diminuisce notevolmente, e la pressione arteriosa continua la sua lieve e progressiva discesa. Più avanti i movimenti respiratori vanno diminuendo in frequenza ed ampiezza e la pressione continua ad abbassarsi lievemente prima, fortemente poi. Finalmente il respiro si arresta e la pressione continua a diminuire tendendo più rapidamente allo zero. Ma quando il respiro si arresta il cuore continua a pulsare sempre più debolmente per qualche tempo ancora.

Dalle due esperienze ora riportate risulta che l'attività respiratoria viene assai modificata dalle diverse dosi del farmaco: eccitata in un primo momento per le piccole velocità d'inoculazione fino a toccare il massimo, raggiunto questo, si ha poi una diminuzione progressiva dell'attività stessa, diminuzione che culmina con un arresto definitivo del respiro. La pressione arteriosa, dopo un aumento iniziale di breve durata, anch'esso dimostrabile soltanto per le piccole velocità d'inoculazione, incomincia a diminuire e tale diminuzione si esagera quando il respiro sta per arrestarsi così che la pressione si riduce moltissimo quando il respiro si arresta. Il cuore continua a pulsare ancora quando il respiro è del tutto arrestato.

Fig. 3 (Tracciati 1° e 2°). - Azione del gluconato di sodio sulla pressione arteriosa e sul respiro del coniglio (Esperienza II).

1° Tracciato. 1° segno.: si incomincia ad inoculare nella vena giugulare destra una soluzione N di gluconato di sodio alla velocità di cc 1 per Kg. e per minuto.

2° Tracciato, 1° segno: si raddoppia la velocità di iniezione.

2° segno: si sospende l'inoculazione del farmaco. Complessivamente sono stati inoculati cc 40,30 di soluzione per Kg., corrispondenti a g. 8,7854, cioè a g-eq. 0,0403 di gluconato di sodio per Kg.

Segnale del tempo ogni 5".

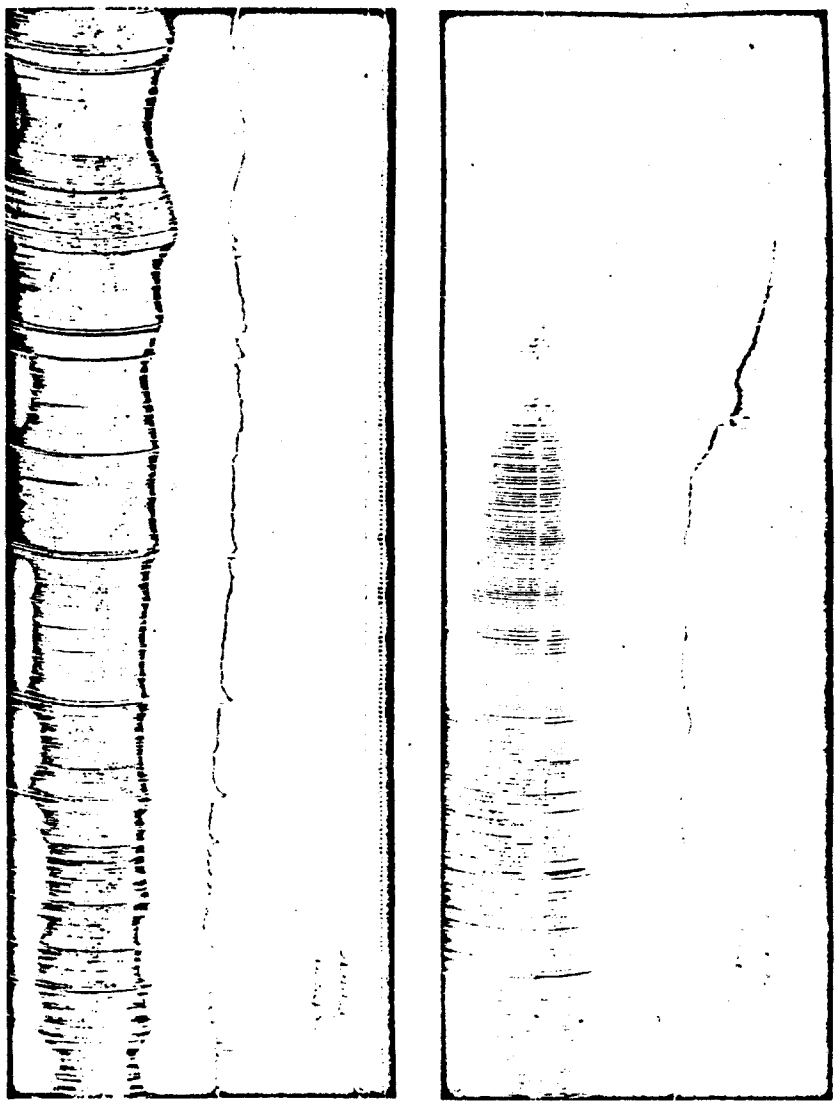

Fig. 3.

(Tracciati 1° e 2°). - Azione del gluconato di sodio sulla pressione arteriosa e sul respiro del coniglio (Esperienza II).

In conclusione, queste esperienze confermano quanto abbiamo visto in principio a proposito dello studio dell'azione generale del farmaco sul coniglio. Osservammo allora che l'attività cardiaca è poco modificata, mentre alterazioni più evidenti risultano a carico della funzione respiratoria. Queste esperienze confermano, dunque, quelle. Sono a carico del respiro le modificazioni più intense e chiare. Quando già appare un aumento notevole dell'attività respiratoria, la pressione presenta un aumento affatto trascurabile, e mentre le modificazioni nell'ampiezza e frequenza del respiro diventano notevolissime, la pressione arteriosa accusa una lieve, progressiva diminuzione ben lontana dai cospicui mutamenti del respiro. Solamente quando l'attività respiratoria subisce una diminuzione assai forte, la pressione arteriosa si abbassa di colpo in modo cospicuo e tende allo zero. Ma quando già il respiro è cessato noi assistiamo al perdurare dell'attività cardiaca che solo qualche minuto dopo si arresta definitivamente.

III. — CONCLUSIONI.

In base alle indagini svolte sulla farmacologia del gluconato di sodio si può affermare che:

1°) la dose minima letale lontana per via endovenosa di gluconato di sodio è, nel coniglio, di g. 7,630 (= g.-eq. 0,035) per Kg. di peso corporeo;

2°) la morte dell'animale superiore (coniglio) avviene con un indebolimento progressivo delle forze per l'azione depressiva esercitata dal farmaco sul sistema nervoso centrale;

3°) mentre le soluzioni a concentrazioni basse del farmaco (da g. 2,18 : 10 milioni a g. 2.18 : 10.000) sono indifferenti sull'attività del cuore isolato di rospo, è dimostrabile un'azione eccitante solamente per soluzioni alquanto concentrate (g. 2,18 : 1.000). Soluzioni molto più concentrate riescono ad arrestarlo definitivamente;

4°) l'azione del gluconato di sodio sui vasi isolati del treno posteriore di rospo (preparazione alla LÄWEN-TRENDELENBURG) è nettamente dilatatrice e solo con le dosi molto alte il farmaco tende a riportare il calibro vasale in condizioni assai simili alle fisiologiche;

5°) l'attività respiratoria del coniglio viene in genere esaltata dalla somministrazione per via endovenosa di gluconato di sodio. Con velocità di iniezione adatte si può mettere in evidenza un'attività eccitatrice sul respiro assai notevole la quale può accrescere enormemente l'attività dell'apparato respiratorio. A questo periodo di intensa eccitazione segue un periodo depressivo che porta alla paralisi della funzione respiratoria;

6°) la pressione sanguigna subisce in principio, per dosi non molto forti del farmaco, un lieve aumento, cui segue una diminuzione progressiva che tende a portare la pressione allo zero. Quando il respiro tende ad arrestarsi, la pressione subisce una diminuzione brusca; allorchè il respiro si arresta, quella subisce un'ulteriore diminuzione, mentre il cuore continua a pulsare ancora per qualche minuto.

RIASSUNTO

L'A. dimostra che nel coniglio la dose minima letale lontana, per via endovenosa, del gluconato di sodio è di g. 7,630 (= g.-eq. 0,035) per Kg. di p. c. e che la morte dell'animale avviene con progressiva diminuzione delle forze per l'azione depressiva esercitata dal farmaco sul sistema nervoso centrale. Sul cuore isolato di rospo il gluconato di sodio ha debole azione eccitante solo in soluzioni alquanto concentrate, mentre soluzioni molto concentrate l'arrestano definitivamente. Sui vasi isolati del treno posteriore di rospo il farmaco dimostra azione sempre cospicuamente dilatatrice, eccetto che per le concentrazioni molto alte, le quali tendono a ricondurre il calibro vasale ai valori iniziali. Sul respiro il farmaco possiede azione nettamente eccitante per dosi adatte; dosi più elevate esplicano azione depressiva sul respiro ed infine l'arrestano del tutto. La pressione sanguigna aumenta lievemente, dapprincipio, soltanto per dosi non forti del farmaco: poi essa decresce continuamente tendendo allo zero. L'attività cardiaca continua per qualche minuto dopo l'arresto del respiro.

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By Siegwart Hermann and Margot Zentner #4

Acid effects and fate of acids in the organism

Report IV:

Determination of the hydrogen ion concentration in the urine and in flowing rabbit blood after oral and intravenous supply of calcium and sodium salts

By Siegwart Hermann and Margot Zentner

Text includes 2 figures

(Received June 10, 1938)

In the preceding reports (1) the remarkable fact was reported that chemically defined acids do not always behave as such physiologically, but that, depending on their kind, they produce acid or alkaline reactions of the body fluids. Free acetic acid, for example, which chemically is clearly of an acid nature, has a strongly alkalizing effect both after intravenous and after oral application. Its sodium salt has no marked effect on venous rabbit blood after intravenous injection. When it is given by mouth, an alkalization of the blood and urine occurs, due to the bacterial degradation of the sodium gluconate to acetic acid, lactic acid, propionic acid, butyric acid and formic acid taking place in the intestine, as these acids, or respectively their sodium salts, alkalize the blood, as we were able to show. Our studies have clearly shown that it is not immaterial whether an acid is given in its free form or as a salt. It was generally held that especially for oral administration it did not matter much whether one or the other form was used. It was considered that the salts are decomposed by the strong gastric hydrochloric acid and that in the intestine salts form again through the alkali present. This, however, can be true only for exceedingly small doses. But if it is a matter of causing acid effects for therapeutic purposes, be it to mobilize the body's mineral salts or to acidify certain organs, e.g. the urinary passages, it is imperative to administer free acids which are of an acid nature also physiologically. It is, therefore, not all the same whether a free acid

or its salt is used. From a certain point of view, the sodium salts investigated by us until now may be termed fairly indifferent. But when the intention is to use salts whose cation is to be therapeutically effective, as is the case for example with calcium salts, it appears desirable in several respects to learn something about the influence exerted by various calcium acid compounds on the hydrogen ion concentration of the body fluids.

I. Variations of the blood and urine pH by calcium chloride

A n/8 solution of calcium chloride was injected at intervals into the Vena jugularis of rabbits by the method described in our earlier reports (loc. cit. 1), and the hydrogen ion concentration was measured in the manner also previously described according to v. Brehmer's intravital method. Portions of 10 cc were injected continuously within 3 minutes. The interval between injections was 15-30 minutes. The lethal dose accordingly was 50-150 cc per kg of animal, depending on the test arrangement. The pH of an n/8 calcium chloride solution is about 5.5. We expected accordingly that the hydrogen ion concentration of the venous blood would be increased. But as can be seen from the curve of figure 1, the very opposite is the case. The blood pH increases, and accordingly the hydrogen ion concentration becomes less. Thus there occurs an unexpected alkalization of the venous blood (5 experiments).

Fig. 1.

If the same quantities of n/8 calcium chloride are allowed to flow into the esophagus at the same intervals of time by means of a dropping funnel, and if one measures at different times the hydrogen ion concentration of the venous blood, it is strange to note that there results also a slight alkalization of the blood, as can likewise be seen from fig. 1 (3 experiments).

During the intravenous and oral application, the urine was taken at different times with a catheter and its pH was measured. Besides, 5 rabbits were fed n/8 calcium chloride, and the pH of the excreted urine was determined. After intravenous as well as after oral administration of calcium chloride a slight alkalization of the urine occurred. The respective values appear in

Tables 1 and 2.

Table 1. Variation of the rabbit urine pH after intravenous supply of various salts.

Salt	cc/kg	Initial pH	Mean	Final pH	Mean
n/4 Ca gluconate	45	5.55	5.50	6.00	5.50
	76	5.15		5.20	
	22	5.15		4.80	
	30	6.35		6.00	
n/8 Ca gluconate	50	5.65		4.70	
n/8 Ca chloride	50	5.05	5.70	6.00	6.50
	55	5.35		6.80	
	63	5.30		6.25	
	150	5.30		5.55	
	90	7.50		7.90	
n/2 Na gluconate	60	5.00	5.20	4.85	4.95
	82	5.40		5.05	
n/8 Na gluconate	30	5.40	5.43	4.45	4.47
	26	5.45		4.50	

Table 2. Variation of the rabbit urine pH after oral supply of various salts.

Salt	cc/kg	Initial pH	Mean	Final pH	Mean
n/4 Calcium gluconate	63	5.15	5.19	7.70	6.81
	73	5.25		6.60	
	20	5.15		5.80	
	15.7	5.20		6.05	
n/8 Calcium chloride	45	5.35	5.30	5.65	6.24
	45	5.60		6.65	
	30	5.10		5.95	
	95	5.15		6.71	
n/2 Sodium gluconate	10	5.90	5.70	6.20	8.18
	10	5.60		8.80	
	10	5.60		9.55	

II. Variations of the blood and urine pH by calcium gluconate

We had noted earlier already that sodium gluconate injected intravenously behaves according to its own pH (6.05) and causes no essential alterations of the venous blood. After injecting n/4 calcium gluconate, whose pH is 6.7, theoretically one should expect no variation in the hydrogen ion concentration of the venous blood. In reality, however, as can be seen from fig. 2, it decreases. Therefore, venous rabbit blood is slightly alkalized by intravenous injection of calcium gluconate (8 experiments).

In Report III (loc. cit.) we found as reason for the blood and urine alkalization caused by oral application of sodium gluconate the bacterial degradation of sodium gluconate in the intestine to acetic acid, lactic acid, propionic acid and formic acid. It was to be expected, therefore, that calcium gluconate would behave similarly. As can be seen from fig. 2, the blood pH is indeed increased (alkalized) after oral supply of calcium gluconate (5 experiments). To see if the degradation of the calcium gluconate by intestinal bacteria can indeed be held responsible for the higher blood pH after oral administration, we inoculated n/8 calcium gluconate solutions with pieces of small intestine and colon in some tubes, incubating them at 37°C. Acidification occurred. To prevent suppression of the bacterial activity by the acid formed, we neutralized about five times during the incubation time. For the neutralization we purposely used sodium carbonate instead of calcium carbonate, in order to simulate the natural conditions in the intestine. The analyses were carried out after 20 days. In two tubes we found no acetic acid at all, in two other tubes traces, and only in one tube acetic acid was clearly identifiable. Formic acid, propionic acid, lactic acid and butyric acid were identifiable in all tubes. For oral administration, all degradation products can be held responsible for the alkalization, as we had demonstrated before. Missing, however, is the most strongly alkalizing acetic acid, which,

with the incubation of sodium gluconate performed in the same manner, is present in large quantities, as can be seen from Table 3. The fact of the degradation explains also that the blood alkalization by orally supplied calcium gluconate cannot be attributed exclusively to the resorption of the calcium ion, as would seem to be the case after orally supplied calcium chloride.

Table 3. Bacterial degradation in vitro.

Incubated at 37°C Inoculated with small intestine and colon pieces	pH of the solution			Incubation time in days	Analysis of the distillate
	Start	Max. variation	Neutralized with		
n/8 Na gluconate	7.00	5.1	2n Na_2CO_3	26	Acetica a.: +++; Propionic a.: + Lactic a.: ++; Formic a.: +
n/8 Ca gluconate	6.55	5.0	2n Na_2CO_3	20	Acetic a.: 0 to trace; Propionic a.: (+) Lactic a.: (+) to ++ Formic a.: +; Butyric a.: +

III. Discussion of the test results

As the most remarkable fact resulting from our experiments it should be emphasized that calcium chloride leads to an alkalization of the blood and of the urine in rabbits both after intravenous injection and after oral administration. We know that these findings are at variance with a generally recognized affirmation according to which calcium chloride is regarded as an acidifying agent. Calcium chloride is recommended in the literature to eliminate alkalosis and the tetany it causes (2). The respective publications actually refer to humans, that is, to omnivores, whereas our findings concern only the plant-eating rabbit. To be able to take a stand on this problem, we must wait for the outcome of our experiments on dogs. For the present we shall discuss only the test results on rabbits. As has been mentioned, the intravenous injection of calcium chloride leads to an alkalization of the urine, while intravenously injected n/4 calcium gluconate hardly changes the hydrogen ion concentration; n/8 Ca gluconate, however, acidifies. That after orally sup-

plied calcium gluconate the urine becomes more alkaline is undoubtedly attributable not only to the resorbed calcium ion, but also to the alkalizing degradation products of gluconic acid produced by intestinal bacteria. Calcium chloride given by mouth cannot be altered by intestinal bacteria. The reduction of the hydrogen ion concentration of the blood, that is, the alkalization after orally supplied calcium chloride, would seem to be a result of the resorbed calcium ion or respectively of the conversion that has occurred in the body fluids. It is noteworthy that after intravenous as well as oral calcium chloride supply the urine pH adjusts itself to approximately the same value. It appears, therefore, that after both intravenous and oral administration, calcium chloride forms compounds with proteins, phosphates and carbonates, which are of importance for the change of reaction toward the alkaline side of the blood and urine. In rabbits (herbivores), alkali chbride) is formed in the body fluids by reaction of calcium chloride with phosphates, protein, etc., and alkali gluconate by reaction of the calcium gluconate. The alkali chloride formed corresponds at most to a 0.73% sodium chloride solution, which after intravenous injection remains almost without influence on the urine. Only after i.v. injection of 100 cc of a n/8 sodium chloride solution (0.73%) did the urine pH decrease from 5.4 to 5.2. The situation is different, however, with intravenously supplied n/4 and especially n/8 sodium gluconate which, as our experiments have shown, increases the urine hydrogen ion concentration. When calcium gluconate is injected i.v., it must be assumed that the gluconic acid component bound to calcium or respectively the sodium gluconate formed by reaction suppresses the alkalizing effect of the calcium compounds formed in the body on the urine, so that after intravenously injected calcium gluconate the urine pH either remains unchanged or decreases, depending on the concentration. Intravenous injection of calcium chloride, instead, causes an alkaline reaction. The alkali chloride forming

by reaction is unable, as we have said before, to exert a neutralizing effect, so that after orally and intravenously supplied calcium chloride the shift toward the alkaline side due to formation of alkaline calcium compounds persists. We must affirm, therefore, that in the rabbit intravenously injected calcium chloride as well as calcium gluconate shift the blood pH toward the alkaline side, and that the urine reaction after calcium chloride also becomes more alkaline, but that calcium gluconate does not shift the urine reaction in alkaline direction due to the neutralizing effect of the gluconic acid component (anion effect). Orally supplied calcium chloride and calcium gluconate shift the reaction of the blood and urine in alkaline direction. When the calcium gluconate is supplied orally, the neutralizing effect of the gluconic acid component does not appear, because with the oral administration of the calcium gluconate the gluconic acid component is destroyed by the bacterial activity in the intestine, since from it other organic acids having a physiologically alkalizing effect are formed. In the carnivore the situation should be somewhat different due to the formation of ammonium chloride or ammonium gluconate. These experiments are not yet completed.

Summary

1. Intravenously or orally supplied calcium chloride reduces the hydrogen ion concentration of the blood as well as of the urine (alkalizes).

2. Intravenously injected calcium gluconate lowers the hydrogen ion concentration of the blood but leaves that of the urine unchanged due to the neutralizing effect of the gluconic acid component, or leads to acidification. Orally supplied calcium gluconate reduces the hydrogen ion concentration of the blood and urine. With the oral administration, the gluconic acid component is degraded by the intestinal bacteria to organic acids, which alkalize physiologically.

3. Intravenously injected sodium gluconate increases the hydrogen ion

concentration of the urine. Orally administered sodium gluconate reduces the hydrogen ion concentration of the urine due to intestinal bacterial activity (alkalizes).

4. The bacterial degradation of the calcium gluconate by intestinal bacteria leads to lactic acid, formic acid and butyric acid and differs from the bacterial degradation of sodium gluconate by the absence of acetic acid.

5. At variance with the views set down in the literature, calcium chloride not only does not cause acidification, but causes alkalization. It should be stressed, however, that the data contained in the literature relate to tests on man and not, as in our case, to herbivores. Experiments on carnivores are being made by us.

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Translated by Carl Demrick Associates, Inc./LH/db

Gluc.

Aus dem Privatforschungsinstitut in Prag. (Vorstand: Dr. Siegwart Hermann, Privatdozent der Deutschen Universität in Prag.)

Säurewirkungen und Säureschicksal im Organismus.

IV. Mitteilung:

Bestimmung der Wasserstoffionenkonzentration im Harn und im strömenden Kaninchenblut nach peroraler und intravenöser Zufuhr von Calcium- und Natriumsalzen.

Von

Siegwart Hermann und Margot Zentner.

Mit 2 Textabbildungen.

(Eingegangen am 10. Juni 1938.)

In den vorangehenden Mitteilungen[1] wurde über die bemerkenswerte Tatsache berichtet, daß chemisch definierte Säuren sich physiologisch nicht immer als solche verhalten, sondern daß sie je nach ihrer Art saure oder alkalische Reaktionen der Körperflüssigkeiten hervorrufen. So wirkt z. B. freie Essigsäure, die chemisch eindeutigen Säurecharakter hat, sowohl nach intravenöser als auch nach oraler Applikation stark alkalisierend. Freie Gluconsäure säuert nach allen Applikationsarten. Ihr Natriumsalz hat nach intravenöser Injektion keine wesentliche Wirkung auf das venöse Kaninchenblut. Wird es per os verabreicht, so tritt infolge des im Darm stattfindenden bakteriellen Abbaues des Natriumgluconats zu Essigsäure, Milchsäure, Propionsäure, Buttersäure und Ameisensäure eine Alkalisierung des Blutes und des Harnes ein, da diese Säuren bzw. ihre Natriumsalze, wie wir zeigen konnten, das Blut alkalisieren. Unsere Untersuchungen haben deutlich gezeigt, daß es nicht gleichgültig ist, ob eine Säure in ihrer freien Form oder als Salz verabreicht wird. Im allgemeinen war man der Meinung, daß es insbesondere für die orale Darreichung mehr oder weniger gleichgültig sei, ob die eine oder die andere Form verwendet wird. Man ging dabei von der Vorstellung aus, daß die Salze durch die starke Magensalzsäure zerlegt werden und daß sich im Darm durch das vorhandene Alkali wieder Salze bilden. Dies kann jedoch nur sehr bedingt für außerordentlich kleine Dosierungen gelten. Handelt es sich aber darum, Säurewirkungen zum Zwecke der therapeutischen Beeinflussung, sei es zur Mobilisierung der Körpermineralsalze oder zur Säuerung bestimmter Organe, z. B. der Harnwege, hervorzurufen, so ist es unbedingt notwendig, freie Säuren zu verabreichen, die auch physiologischen Säurecharakter haben. Es ist also

[1] Hermann, Siegwart, Richard Neiger u. Margot Zentner: Naunyn-Schmiedebergs Arch. 188, 526—537 (1938) I. u. II. Mitteilung; III. Mitteilung 189, 539—546 (1938).

bisher untersuchten Natriumsalze können von einem gewissen Grade
kte als einigermaßen indifferent bezeichnet werden. Handelt es sich
darum, Salze verwenden, deren Kation therapeutisch wirksam
soll, wie dies z. B. bei Calciumsalzen der Fall ist, so erscheint es in
icher Hinsicht wünschenswert, etwas über den Einfluß auf die Wasser-
ffionenkonzentration der Körpersäfte zu erfahren, den verschiedene
ium-Säure-Verbindungen ausüben.

I. Änderungen des Blut- und Harn-p_H durch Calciumchlorid.

Es wurde eine n/8 Lösung von Calciumchlorid nach der in unseren
ieren Mitteilungen (l. c. 1) beschriebenen Methodik in die Vena jugularis
Kaninchen in Intervallen injiziert und die Wasserstoffionenkonzen-
ion in der gleichfalls früher beschriebenen Weise nach der intravitalen

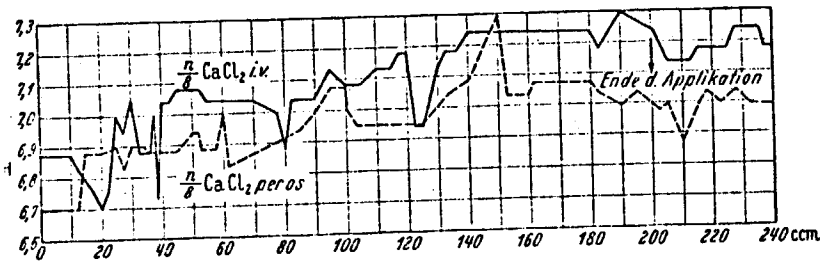

Abb. 1.

thode von v. Brehmer gemessen. Injiziert wurden je 10 ccm konti-
erlich innerhalb 3 Minuten. Der Abstand von einer Injektion zur anderen
rug 15—30 Minuten. Die tödliche Dosis war dementsprechend je nach
Versuchsanordnung 50—150 ccm pro kg Tier. Das p_H einer n/8 Calcium-
oridlösung beträgt etwa 5,5. Danach haben wir erwartet, daß die
sserstoffionenkonzentration des venösen Blutes erhöht werden wird.
e aus der Kurve der Abb. 1 zu ersehen ist, tritt aber gerade das Gegenteil
. Das Blut-p_H steigt an, die Wasserstoffionenkonzentration wird dem-
ch geringer. Es tritt also eine unerwartete Alkalisierung des venösen
utes ein (5 Versuche).

Läßt man die gleichen Mengen n/8 Calciumchlorid in gleichen Zeit-
ständen mittels Tropftrichter in den Oesophagus einfließen und mißt in
rschiedenen Zeitpunkten die Wasserstoffionenkonzentration des venösen
utes, so ergibt sich, wie gleichfalls aus der Abb. 1 hervorgeht, sonder-
rerweise auch eine leichte Blutalkalisierung (3 Versuche).

Während der intravenösen und oralen Applikation wurde der Harn
ittels Katheter in verschiedenen Zeitpunkten entnommen und sein p_H
messen. Außerdem wurden 5 Kaninchen mit n/8 Calciumchlorid ge-
ttert und das p_H des abgedrückten Harnes bestimmt. Sowohl nach der
aus den Tabellen 1 und 2 zu ersehen.

Tabelle 1. Änderung des Kaninchenharn-p_H nach intravenöse ufuhr verschiedener Salze.

Salz	Anzahl der ccm/kg	Anfangs-p_H Mittelwert		End-p_H Mittelwert	
n 4 Ca-Gluconat	45	5,55	5,50	6,00	5,50
	76	5,15		5,20	
	22	5,15		4,80	
	30	6,35		6,00	
n/8 Ca-Gluconat	50	5,65		4,70	
	50	5,05		6,00	
n/8 Ca-Chlorid	55	5,35	5,70	6,80	6,50
	63	5,30		6,25	
	150	5,30		5,55	
	90	7,50		7,90	
n/2 Na-Gluconat	60	5,00	5,20	4,85	4,95
	82	5,40		5,05	
n/8 Na-Gluconat	30	5,40	5,43	4,45	4,47
	26	5,45		4,50	

Tabelle 2. Änderung des Kaninchenharn-p_H nach peroraler Zufuhr verschiedener Salze.

Salz	Anzahl der ccm/kg	Anfangs-p_H Mittelwert		End-p_H Mittelwert	
n/4 Calciumgluconat ...	63	5,15	5,19	7,70	6,81
	73	5,25		7,70	
	20	5,15		5,80	
	15,7	5,20		6,05	
n/8 Calciumchlorid	45	5,35	5,30	5,65	6,24
	45	5,60		6,65	
	30	5,10		5,95	
	95	5,15		6,71	
n/2 Natriumgluconat ...	10	5,90	5,70	6,20	8,18
	10	5,60		8,80	
	10	5,60		9,55	

II. Änderungen des Blut- und Harn-p_H durch Calciumgluconat.

Wir haben bereits früher festgestellt, daß sich intravenös injiziertes Natriumgluconat seinem eigenen p_H (6,05) gemäß verhält und keine wesentlichen Änderungen des venösen Blutes herbeiführt. Injiziert man n/4 Calciumgluconat, dessen p_H 6,7 beträgt, so wäre theoretisch keine Veränderung der Wasserstoffionenkonzentration des venösen Blutes zu erwarten. In Wirklichkeit nimmt sie aber wie aus der Abb. 2 zu ersehen ist, ab. Das venöse Kaninchenblut wird also durch intravenöse Injektion von Calciumgluconat schwach alkalisiert (8 Versuche).

In der I. c. III. Mitteilung konnten wir als Grund der durch orale Applikation von Natriumgluconat hervorgerufenen Blut- und Harnalkalisierung den bakteriellen Abbau des Natriumgluconats im Darm zu Essigsäure, Milchsäure, Propionsäure und Ameisensäure feststellen. Es war also zu erwarten, daß sich Calciumgluconat in der gleichen Weise verhalten wird. Wie aus der Abb. 2 ersichtlich ist, wird das Blut-p_H nach oraler Zufuhr von Calciumgluconat auch tatsächlich erhöht (alkalisiert) (5 Versuche). Um zu sehen, ob der Abbau des Calciumgluconats durch Darmbakterien tatsächlich für die Blut-p_H-Erhöhung nach oraler Zufuhr verantwortlich gemacht werden kann, haben wir in einigen Kolben n/8 Calciumgluconatlösungen mit Dünn- und Dickdarmstücken beimpft und bei 37° C bebrütet. Es trat Säuerung ein. Damit die Bakterientätigkeit durch die entstandene Säure nicht unterdrückt werde, wurde während der Bebrütungsdauer etwa fünfmal abgestumpft. Für die Neutralisation wurde

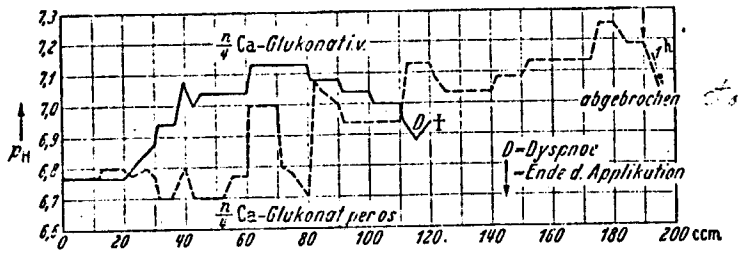

Abb. 2.

anstatt Calciumcarbonat mit Absicht Natriumcarbonat verwendet, um die natürlichen Verhältnisse im Darm einigermaßen nachzuahmen. Nach 20 Tagen wurden die Analysen durchgeführt. Essigsäure war in zwei Kolben überhaupt nicht, in weiteren zwei Kolben in Spuren und nur in einem Kolben deutlich nachweisbar. Ameisensäure, Propionsäure, Milchsäure und Buttersäure konnte in allen Kolben nachgewiesen werden. Alle Abbau-

Tabelle 3. Bakterieller Abbau in vitro.

Bebrütet bei 37° C Mit Dünn- und Dickdarmstücken beimpft	p_H der Lösung: Anfang	p_H der Lösung: Max. Änderung	p_H der Lösung: Abgestumpft mit	Dauer der Bebrütung in Tagen	Analyse des Destillates
n/8 Natriumgluconat ..	7,00	5,1	2 n Na_2CO_3	26	Essigsäure: +++; Propionsäure: + Milchsäure: ++; Ameisensäure: +
n/8 Calciumgluconat ..	6,55	5,0	2 n Na_2CO_3	20	Essigsäure: 0 bis Spur; Propionsäure (+) Milchsäure: (+) bis + + Ameisensäure: +; Buttersäure: +

gewiesen haben, für die Alkalisierung verantwortlich gemacht werden. Es fehlt allerdings die am stärksten alkalisierende Essigsäure bei der in gleicher Weise vorgenommenen Bebrütung des Natriumgluconats in großen Mengen vorhanden ist, wie aus der Tabelle 3 hervorgeht. Die Tatsache des Abbaues erklärt auch, daß die Blutalkalisierung durch oral zugeführtes Calciumgluconat nicht ausschließlich auf Rechnung der Resorption des Calciumions zu setzen ist, wie dies nach oral zugeführten Calciumchlorid der Fall sein dürfte.

III. Besprechung der Versuchsergebnisse.

Als bemerkenswerteste Tatsache, die sich aus unseren Versuchen ergeben hat, ist hervorzuheben, daß Calciumchlorid sowohl nach intravenöser Injektion als auch nach oraler Darreichung bei Kaninchen zu einer Alkalisierung des Blutes und des Harnes führt. Wir wissen, daß wir uns bei diesen Feststellungen im Gegensatz zu einer allgemein anerkannten Behauptung befinden, nach welcher Calciumchlorid als säuerndes Agens angesehen wird. Calciumchlorid wird im Schrifttum zur Beseitigung der Alkalose und der dadurch bedingten Tetanie empfohlen[2]. Die diesbezüglichen Arbeiten beziehen sich allerdings auf Menschen, also auf Omnivoren, während unsere Feststellungen lediglich das pflanzenfressende Kaninchen betreffen. Um zu diesem Fragenkomplex Stellung nehmen zu können, müssen wir erst die Ergebnisse unserer Versuche an Hunden abwarten. Vorläufig wollen wir nur die Versuchsresultate bei Kaninchen besprechen. Wie bereits erwähnt, hat die intravenöse Injektion von Calciumchlorid eine Alkalisierung des Harnes zur Folge, während intravenös injiziertes n/4 Calciumgluconat die Wasserstoffionenkonzentration kaum verändert; n/8 Ca-Gluconat jedoch säuert. Daß der Harn nach oral zugeführtem Calciumgluconat alkalischer wird ist zweifellos nicht nur auf das resorbierte Calciumion zurückzuführen, sondern auch auf die durch Darmbakterien entstandenen alkalisierenden Abbauprodukte der Gluconsäure. Per os verabreichtes Calciumchlorid kann durch Darmbakterien nicht verändert werden. Die Verringerung der Wasserstoffionenkonzentration des Blutes, also die Alkalisierung nach oral zugeführtem Calciumchlorid, dürfte eine Folge des resorbierten Calciumions bzw. der in den Körpersäften erfolgten Umsetzung sein. Es ist bemerkenswert, daß sich das Harn-p_H sowohl nach intravenös als auch nach oral zugeführtem Calciumchlorid ungefähr auf den gleichen Wert einstellt. Calciumchlorid dürfte somit nach intravenöser als auch nach oraler Verabreichung Verbindungen mit Eiweißkörpern, Phosphaten und Carbonaten eingehen, welche für die Reaktionsänderung nach der alkalischen Seite des Blutes und des Harnes von Bedeutung sind.

[2] György: Siehe Literaturzusammenstellung: Starkenstein: Handb. d. Physiologie 13, 398 (1929).

i Kaninchen (Herbivoren) bildet sich in den Körpersäften durch Umzung des Calciumchlorids mit Phosphaten, Eiweiß usw. Alkalichlorid d durch Umsetzung des Calciumgluconats Alkaligluconat. Das gebildete kalichlorid entspricht höchstens einer 0,73%igen Natriumchloridlösung, lche nach intravenöser Injektion auf den Harn fast ohne Einfluß bleibt. st nach i. v. Injektion von 100 ccm einer n/8 Natriumchloridlösung 73%) ging das Harn-p_H von 5,4 auf 5,2 zurück. Anders verhält sich er intravenös zugeführtes n/4 und vor allem n/8 Natriumgluconat, das, e unsere Versuche ergeben haben, die Harn-Wasserstoffionenkonzenation erhöht. Wird Calciumgluconat i. v. injiziert, so ist anzunehmen, ß durch den an Calcium gebundenen Gluconsäureanteil bzw. das rch Umsetzung entstandene Natriumgluconat die alkalisierende Wirng der im Körper entstandenen Calciumverbindungen auf den Harn ralysiert wird, so daß nach intravenös injiziertem Calciumgluconat s Harn-p_H je nach der Konzentration entweder unverändert bleibt, ler absinkt. Die intravenöse Injektion von Calciumchlorid hat hingen eine alkalische Reaktion zur Folge. Das sich durch Umsetzung ldende Alkalichlorid vermag, wie wir bereits gesagt haben, keine neutralierende Wirkung auszuüben, so daß nach oral und intravenös zugeführtem lciumchlorid die Reaktionsverschiebung infolge Bildung alkalischer alciumverbindungen nach der alkalischen Seite bestehen bleibt. Wir müssen so festhalten, daß beim Kaninchen sowohl intravenös injiziertes alciumchlorid als auch Calciumgluconat das Blut-p_H nach der alkalichen Seite verschieben und daß die Harnreaktion nach Calciumhlorid gleichfalls alkalischer wird, daß aber Calciumgluconat die arnreaktion infolge der neutralisierenden Wirkung des gluconsauren nteiles (Anionenwirkung) nicht nach der alkalischen Richtung erschiebt. Oral zugeführtes Calciumchlorid und Calciumgluconat verhieben die Reaktion des Blutes und des Harnes nach der alkalischen ichtung. Bei oraler Zufuhr tritt beim Calciumgluconat die neutralierende Wirkung des Gluconsäureanteiles nicht in Erscheinung, weil bei er oralen Darreichung des Calciumgluconats der Gluconsäureanteil durch ie Bakterientätigkeit im Darm vernichtet wird, da sich aus ihm andere rganische Säuren mit physiologisch alkalisierender Wirkung bilden. Beim leischfresser dürften die Verhältnisse infolge der Bildung von Ammonhlorid bzw. Ammongluconat etwas anders liegen. Diese Versuche sind och nicht beendet.

Zusammenfassung.

1. Intravenös oder oral zugeführtes Calciumchlorid setzt sowohl die Vasserstoffionenkonzentration des Blutes als auch die des Harns herab alkalisiert).

2. Intravenös injiziertes Calciumgluconat vermindert die Wasserstoffionenkonzentration des Blutes, läßt aber die des Harns infolge der neutralisierenden Wirkung des Gluconsäureanteils unverändert oder führt zur Säuerung. Oral zugeführtes Calciumgluconat setzt die Wasserstoffionenkonzentration des Blutes und des Harns herab. Bei der oralen Darreichung wird der Gluconsäureanteil von den Darmbakterien zu organischen Säuren abgebaut, welche physiologisch alkalisieren.

3. Intravenös injiziertes Natriumgluconat erhöht die Wasserstoffionenkonzentration des Harns. Per os verabreichtes Natriumgluconat vermindert die Wasserstoffionenkonzentration des Harns infolge der Darmbakterientätigkeit (alkalisiert).

4. Der bakterielle Abbau des Calciumgluconats durch Darmbakterien führt zu Milchsäure, Ameisensäure und Buttersäure und unterscheidet sich von dem bakteriellen Abbau des Natriumgluconats durch das Fehlen von Essigsäure.

5. Im Gegensatz zu den im Schrifttum niedergelegten Anschauungen bewirkt Calciumchlorid nicht nur keine Säuerung, sondern eine Alkalisierung. Es ist jedoch zu betonen, daß sich die in der Literatur befindlichen Angaben auf Versuche beim Menschen und nicht, wie in unserem Falle, auf Pflanzenfresser beziehen. Versuche an Fleischfressern werden von uns angestellt.

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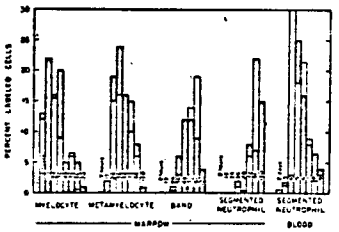

FIG. 2. Time relationship between inj. of tritiated thymidine and appearance of label in the various cells of the neutrophil series. (Hatched bars, male beagle; open bars, female beagle.)

during aspiration, which influences the percentage of labeled segmented neutrophils in marrow. When this factor is considered, it is clear that the true peak in marrow occurs about a day earlier than in the periphery. A similar sequence of segmented neutrophils in marrow and blood has been seen in the mouse with C^{14}-labeled adenine(5). It is of interest to note that labeled myelocytes may be detectable in marrow for several days. It can be inferred from this that some labeled neutrophils are released to the circulation for about an equivalent length of time, although in decreasing numbers. This conclusion is supported by an apparent decrease in grain counts of the peripheral neutrophils with time. The overall sequence of events is depicted in Fig. 2. Comparable results were obtained in both dogs.

There is, in general, a reasonable correspondence between these results and the indirect approximations of neutrophil balance reported previously(6,7). It would appear from these data that the time for differentiation of a myelocyte to a segmented neutrophil in the dog is between 2 and 3 days. Another 2 to 3 days are spent in the segmented form. More detailed counting and more frequent sampling is necessary, however, to establish the precise chronology of neutrophil maturation and the life cycle of the proliferating elements.

Summary. The pattern of neutrophilic granulocyte development has been studied in 2 dogs by high resolution radioautography with tritiated thymidine. Two to 3 days are required for differentiation from the myelocyte to the segmented neutrophil. The half time for disappearance of the latter in the periphery appears to be of the order of 2 days.

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Effectiveness of Gluconate, Chloride, and Other Sodium Solutions in Treatment of Experimental Burn Shock.* (24191)

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There is adequate experimental(1,2,3) and clinical(4,5,6,7) evidence for the effectiveness of oral sodium solutions in prevention and treatment of burn shock. For other types of shock the experimental evidence(8,9,10,11) is just as good, but clinical confirmation is meager. There is relatively little evidence on optimum electrolyte composition of solutions for oral use. In particular, there has been no systematic approach to the design of an oral sodium solution which will offer a great improvement in palatability with minimal, if any, loss of effectiveness. Palatability of currently employed solutions has been no great problem in hospital use, but it may be of major importance in treatment of burns *en masse*. Acceptability of iced solution in hospital environment might be expected to be better than acceptability of the same solution in tepid water on battlefield or in temporary facilities of a disaster-stricken city. Our study approached the determination of an effective, but more palatable, oral sodium solution by comparing effectiveness of solutions of a number of sodium salts, singly and in various combinations; and by appraising the influence on effectiveness of various ancillary measures for improving palatability.

* Dr. R. Carl Millican and Dr. Sanford M. Rosenthal of N.I.H. made their laboratory available for preliminary experiments. Dr. Stanley Levenson, Walter Reed Army Inst. of Research, furnished encouragement and criticism.

Methods. Mice were subjected to standard thermal injury, and were treated with various sodium solutions. Mortality measured at 24 hours was chosen as criterion of effectiveness of the solutions, after recommendations of Hamilton *et al.*(12), Rosenthal and Millican (13), and Allen(14). The 24 hour point appears to offer a separation between early deaths, due to shock, and late deaths, due to other factors(15). The anions chosen for study included chloride, because it is the experimental standard; bicarbonate, lactate, citrate, and acetate, because they have been used clinically; gluconate, because it is quite palatable;† and succinate, in spite of its impalatability, because it has been used experimentally with recognition of its influence on respiration of damaged tissue(16,17). Solutions were administered in 2 doses; the first given orally, the second either orally or intraperitoneally. Intraperitoneal injection was selected for the second dose as a matter of convenience. Rosenthal(1) and Millican *et al.*(18) found no difference between the effect of oral and intraperitoneal administration. After preliminary experiments, a total dosage of 18% of body weight was selected. This volume approaches the tolerance of animals for rapid oral administration, and a large dose was desired to increase the probability of detecting any deleterious effect from excessive dosage of anions. Female albino mice were lightly anesthetized with ether and immersed to the axilla in water at 70°C. The hair was not clipped, current practice in Rosenthal's laboratory. The mice were of 2 different strains and weights: 1500 were W. R. Bagg mice of 12-14 g; 1215 were CWL mice of 14-18 g. In individual experiments all mice were of same strain, weight, and nutritional status. Period of immersion was adjusted to strain and weight of animals to obtain in untreated animals a 24 hour mortality of 80% to <100%. All W. R. Bagg mice were immersed for 7 seconds. CWL mice were immersed 8 seconds or 8.5 seconds; 0.5 second was added to immersion time for groups of mice which appeared unusually vigorous and active. In 2 experiments (A, Tables I and II) mortality of untreated animals fell outside the range 80% to <100%. These experiments were repeated (B, Tables I and II). For experiments involving W. R. Bagg mice environmental temperature was 23.5°C ± 1.5°; for CWL mice, 22.8°C ± 0.6°. Immediately after immersion, the first dose was administered by gavage. The second dose followed at 2 hours. With 2 exceptions, all solutions contained sodium as the only cation, and in concentration of 140 meq/l. The Lactated Ringer's Solution was the USP product, and one of the multiple ion formulations contained 145 meq/l of sodium, the additional 5 meq/l being in a flavoring agent. Confidence limits (95%) of percentage mortality were computed by the formula:

$$\text{C.L.} = p \pm \left(t_{.05} \times \sqrt{\frac{pq}{n}} \right).$$

Probability (p) figures for validity of differences in mortality were obtained from standard t tables, with t computed by the formula:

† Dr. Joseph M. White and Miss R. Millard called my attention to the palatability of sodium gluconate.

$$t_z = \frac{p_1 - p_2}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}}.$$

All p values quoted are for mathematically independent comparisons of data involving the same immersion times, and animals of same strain, weight, and nutritional status.

Results. Standard solutions. Following treatment with sodium chloride, Lactated Ringer's Solution, and the customary (6,7,19) chloride-bicarbonate mixture, mortality was approximately the same (Table III). Mortality following treatment with sodium chloride is much higher than that reported by Millican *et al.* (2) in similar, but not exactly comparable, experiments. "However, the significant criterion is the deviation from control which occurs as a result of treatment . . ." (20).

TABLE I. Mortality following Treatment with Lactate and Chloride as Related to Untreated Mortality.

Solution*						
Chloride	None	140	105	70	35	
Lactate	None		35	70	105	140
Exp. A						
†*	(.70)	(.23)	.33	.33	.13	.33
n	(30)	(30)	30	30	30	30
Exp. B						
†	(.97)	(.55)	.73	.43	.67	.77
n	30	30	30	30	30	30‡

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% body wt by gavage, 12% intraper.
( ) = Data utilized in Table III.
n = No. of animals.
* Figures = Anions in meq/l.
† Mortality at 24 hr.
‡ Tetany observed.

TABLE II. Mortality following Treatment with Chloride and Gluconate if Injury Is Equal to or Greater Than LD_{100}.

Solution*										
Chloride	None	140	122.5	105	87.5	70	52.5	35	17.5	
Gluconate	None		17.5	35	52.5	70	87.5	105	122.5	140
Exp. A										
†	1.00	.27	.47	.20	.67	.60	.73	.67	.93	.93
n	30	15	15	15	15	15	15	15	15	15
Exp. B										
†	.93	.13	.07	.13	.07	.13	.07	.07	.13	.47
n	15	15	15	15	15	15	15	15	15	15

Strain, CWL: wt, 14-18 g; immersion: A, 8.5 sec.; B, 8 sec.; method of admin., 6% body wt by gavage, 12% intraper.
* Figures = Anions in meq/l. † Mortality at 24 hr. n = No. of animals.

TABLE III. Mortality following Treatment with Standard Solutions.

Solution*	None	Cl 140	Cl 93 HCO_3 47	Lactated Ringer's
†	.84	.28	.24	.31
.95 C.L.	± .033	± .041	± .039	± .042
n	120	120	120	120

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% body wt by gavage, 12% intraper.
n = No. of animals.
.95 C. L. = 95% confidence limits.
* Figures = Anions in meq/l.
† Mortality at 24 hr.

Single anions in concentration of 140 meq/l. Under our conditions, some anions (lactate, acetate, bicarbonate, citrate) are far less effective than chloride in lowering mortality below that of the control (untreated) group (Table IV). That this lack of effectiveness may be the result of active toxicity is suggested by appearance of tetany in some groups of animals treated with these anions alone or in high concentration with chloride (Tables IV, V). Tetany was never seen in animals treated with gluconate, and only rarely with succinate. The number of animals used in simultaneous comparison, presented in Table IV, is too small to give validity to differences between lactate, acetate, bicarbonate, and citrate, which showed a mortality range of 77-97%. Succinate was significantly more effective than lactate ($p < .02$). In this experiment no valid difference between gluconate and chloride is demonstrated. However, when total experience with 140 meq/l gluconate solution is compared with experience with 140 meq/l chloride solution, the lesser effectiveness of gluconate is more striking: Cl 23% (n = 90), Gluc 51% (n = 90), no treatment 93% (n = 105): Cl *vs.* Gluc $p < .001$.

TABLE IV. Mortality following Treatment with Various Anions, 140 meq/l.

Solution	None	Cl	Glue	Succ	Lact	Acet	HCO_3	Citr	Lactated Ringer's
†	(.93)	(.27)	.40	.47	.77	.77	.87	.97	(.40
n	(30)	(30)	30	30*	30*	30*	30	30	(30

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% of body wt by gavage, 12% intraper. ( ) = Data utilized in Table III.
n = No. of animals.
* Tetany observed. † Mortality at 24 hr.

Mixtures of chloride and other anions. In spite of the relative ineffectiveness of non-chloride anions of sodium when used alone, the results from mixtures of these anions with chloride (Table V) indicate that potency of chloride is generally retained even when it is diluted half and half with a non-chloride anion. The data on gluconate-chloride mixtures (Tables II, V) indicate that even three-quarters of the chloride in a 150 meq/l solution might be replaced with gluconate without pronounced loss of effectiveness.

Effect of citrate, and free citric acid in mixtures. It was anticipated that citrate or free citric acid or both might prove deleterious on parenteral injection. Consequently, tests for effectiveness of some formulations containing citrate and citric acid included both oral and intraperitoneal administration. Citrate in concentration of 35 meq/l may be deleterious on intraperitoneal injection, but the mortality data are not conclusive: both doses oral, 20% (n = 30); second dose intraperitoneal, 63% (n = 75). However, these are composite figures from several different experiments. A single paired experiment did not demonstrate a significant difference (oral 40%, intraperitoneal 47%). It is clear that

TABLE V. Composite Mortality following Treatment with Graded Proportions of Chloride and Other Anions.

Solution		None							Lactated Ringer's
Chloride*			140	105	93	70	35		109
Non-chloride anion*				35	47	70	105	140	28
—		.91	.36						
		255	270						
Glue	†			.23		.31	.30	.49	
	n			60		75	60	75	
Succ	†			.53	.50	.33	.43	.55	
	n			60	30	30	30	60‡	
Lact	†			.53	.20	.30	.31	.62	.31
	n			60	30	90	45	90‡	120
Acet	†			.40		.50	.51	.77	
	n			30		60	45	30‡	
HCO_3	†			.37	.29	.43	.53	.87	
	n			60	90	30	15‡	60‡	
Citr	†			.63		.60	.67	.97	
	n			75		30	15‡	30	

Data not suitable for independent mathematical comparisons. Composite results with W. R. Bagg 13 g mice immersed 7 sec., and CWL 16 g mice immersed 8 and 8.5 sec. Method of admin., 6% body wt by gavage, 12% intraper.
n = No. of animals.
* Figures = Anions in meq/l. † Mortality at 24 hr. ‡ Tetany observed.

TABLE VI. Mortality following Treatment with Solutions Containing Free Citric Acid.

Solution*	None	Cl 140	Cl 68, Gluc 46, Citr 26					
Free citric acid, g/l		0	0	1.5	3.0	3.0	4.5	4.5
Method of admin.		P	P	P	P	O	P	O
†	.98	.40	.47	.53	.60	.27	.97	.42
n	60	60	15	15	15	15	60	135

Strain, CWL; wt, 14-18 g; immersion, 8.5 sec.
P = 6% of body wt at 0 hr by gavage; 12% of body wt at 2 hr, intraper. O = 9% of body wt at 0 hr by gavage; 9% of body wt at 2 hr by gavage. n = No. of animals.
* Figures = Anions in meq/l. † Mortality at 24 hr.

citrate is tolerated well at 35 meq/l and less on oral administration (Tables VI, VII). Free citric acid, 4.5 g/l, definitely increases mortality when given intraperitoneally (Table VI). Addition of a sweetening agent, and effervescence in the solution, do not appear to impair effectiveness after oral administration (Table VII).

Discussion. From a pharmaceutical standpoint, it is very difficult to alter, conceal, or compensate for the taste of chloride in concentrations of 90 meq/l or higher. It would be a great gain in palatability if the chloride component of oral sodium solutions could be reduced below this level. Such appears feasible, from the point of view of burn shock prevention and treatment, with the use of several non-chloride ions (acetate, citrate, lactate, gluconate), which in themselves are quite palatable. Of these, the safest appears to be gluconate, since it is the most effective in 140 meq/l solution, and has not shown a toxic effect under our conditions.

Additional gains in palatability can be obtained by effervescence with CO_2, free citric acid, and a sweetening agent.‡ To date, the best compromise I have found between effectiveness in burned mice and subjective palatability to the author's family and neighbors is:§

Sodium chloride	2.0 g
" gluconate	17.4
" bicarbonate	2.1
Citric acid, anhydrous	4.8
Sodium cyclohexylsulfamate	1.0

In one liter of water the solution offers a pleasant taste, an attractive fizz, and the following ionic composition:

Sodium	145 meq/l
Chloride	35
Gluconate	80
Bicarbonate	(converted to citrate)
Citrate	25
Citric acid	47
Cyclohexylsulfamate	5

This solution is comparable in effectiveness to sodium chloride in prevention and treatment of experimental burn shock in mice under our conditions (Table VII: Cl 42% *vs.* USG-20 39%, p = .7). If used in humans in dosage of 15-18% of body weight, the solution would supply approximately 1000 calories. The proportion of normal caloric requirement supplied to mice was considered negligible (2.5%). A solution containing a larger proportion of chloride (Table VI) might stand on better physiological grounds.

Conclusions. 1) Chloride appears to be the most important anion in oral sodium solutions used for prevention and treatment of experimental burn shock in mice. 2) Citrate, acetate, bicarbonate, and lactate are far less effective than chloride, and may be toxic in large doses. 3) There is no sanctity in the customary 93:47 or higher meq ratio between chloride and other ions in oral electrolyte solutions used in these experiments. 4) Gluconate appears to serve as a partial substitute for chloride, and it lends palatability: experimental and clinical studies are indicated on gluconate metabolism in man.

TABLE VII. Mortality following Treatment with Solution Containing Effervescence, Sweetening Agent, and Citric Acid.

Solution*	None	Cl 140	Cl 35, Gluc 80, Citr 25‡	
			P	O
Method of admin.				
†	.91	.42	.70	.39
n	45	60	30	120

Strain, CWL; wt, 14-18 g; immersion, 8.5 sec.
P = 6% of body wt at 0 hr by gavage; 12% of body wt at 2 hr, intraper. O = 9% of body wt at 0 hr by gavage; 9% of body wt at 2 hr by gavage.
n = No. of animals.
* Figures = Anions in meq/l.
† Mortality at 24 hr.
‡ Plus free citric acid, 3.0 g/l, and sodium cyclohexylsulfamate, 5 meq/l; effervescent = citrate formed by interaction of citric acid and $NaHCO_3$.

‡ All such preparations were prepared through the courtesy of Mr. A. W. Taff, Emerson Drug Co.
§ Formulation USG-20, Emerson Drug Co.

This article is not to be construed as reflecting the position of the U. S. Army or any element thereof. The author alone is responsible for data presented and conclusions drawn.

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Reversal of Respiratory Decline in Necrotic Liver Degeneration by Intraportal Antioxidants.[1] (24192)

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A specific metabolic lesion, respiratory decline, has been demonstrated in liver slices from rats maintained on a diet producing necrotic liver degeneration (1,2). Normal-appearing slices of such livers are unable to maintain respiration in the Warburg apparatus after initially normal O_2 consumption for the first half hour. The defect is characteristic for the latent period of the disease; it precedes the acute pathological lesion by several weeks. Respiratory decline is prevented by feeding of those factors which protect against liver necrosis, *i.e.*, cystine†, Vit. E, and Factor 3. The lesion is reversed within minutes after injection of Vit. E into the portal vein (3)‡, but not after that of Factor 3§. Pre-

[1] This manuscript was originally submitted on April 30, 1956.
* These studies were performed during tenure of Brewer's Yeast Council Research Fellowship.

† Since submission of this paper it has been shown that Factor 3 is an organic selenium compound (Schwarz, K., Foltz, C. M., *J. Am. Chem. Soc.*, 1957, v79, 3292). The protective effect of L-cystine is caused by a trace contamination with Factor 3-active selenium.
‡ *In vitro* addition of α-tocopherol to deficient liver slices, either as an emulsion or in water-soluble forms, has no significant effect on metabolic lesion.
§ Relation of Factor 3-active selenium compounds to respiratory decline will be the subject of a separate report.

8

Lack of Evidence for a Thrombolytic Effect of Sodium Gluconate in Arteries

the Department of Hematology and Coagulation (Prof. Dr. T. M. Fliedner) Center of Internal Medicine, University of Ulm (Germany)

H. Rasche, V. Hiemeyer and K. Schniep*

It has been shown that intravascular clots in man and certain animals can be ...olved by the proper activation of the fibrinolytic system, which is possible by use ...reptokinase and urokinase. However, these substances are expensive to produce ...because of their protein character, are associated with occasional side effects. For ...reasons attempts have been made by numerous investigators to produce such ...minogen activators without disadvantages. Among others, von Kaulla (1960) has ...stigated a large number of synthetic compounds which, in vitro, convert plas...ogen to plasmin. Recently Kopper (1966) reported about thrombolysis due to ...ium gluconate. Since we have developed a model to examine thrombolytic activity ...t arteries, it was of interest to compare this compound in our model with the ...t of streptokinase. Although we could not find evidence for a fibrinolytic effect of ...ium gluconate, it appears worthwhile to report on our findings.

Method

The experiments were performed on cats of a bodyweight of 2 to 4 kg. The animals were kept ...r Nembutal (Pentobarbital-Na, Abbot Co.) anaestesia. Both femoral arteries were carefully ...ed in a length of 3 cm. In one of the two femoral arteries a clot was induced distally from the ...tion of the A. profunda femoris in the following way: 1 min after clamping off the vessel with ...Gefässbach clamp" a second clamp was applied exactly 1 cm further below. All the collaterals ...s area were ligated. Ten min later 100 u of thrombin dissolved in 0.1 ml physiological saline ...ipeted into the clamped off segment with a small gage needle. An additional 10 min later, ...proximal clamp was opened momentarily so that blood entered the distally occluded vessel and ...ed with the thrombin solution. Clot-formation occurred within 20–30 min. The clamps were ...ved just before starting the treatment in order to have a 1 cm standard clot. Clot-formation ...r than in the occluded segment was never observed. A polyethylene catheter was introduced ...the contralateral femoral artery directed toward the iliac artery. Through this catheter blood ...ples were withdrawn for laboratory determinations. Angiographic studies were performed ...tedly and sodium gluconate was infused with the aid of a pressure pump.

Control of Thrombolysis

In previous experiments with streptokinase administration, the gradual disappearance of the ...has been observed simply by watching the color of the vessel. As thrombolysis proceeded, the ...mely dark coloration of the vessel became bright red. In order to record the earliest moment ...canalization, control angiograms were performed as soon as exterior changes indicated reopen...of the vessel. In case of persistance of the blockage, the length of the clot was measured after ...death of the animal.

* Substantial parts of the present paper will be used by K. Schniep as dissertation and sub...ted to the Department of Medicine at the University of Ulm.

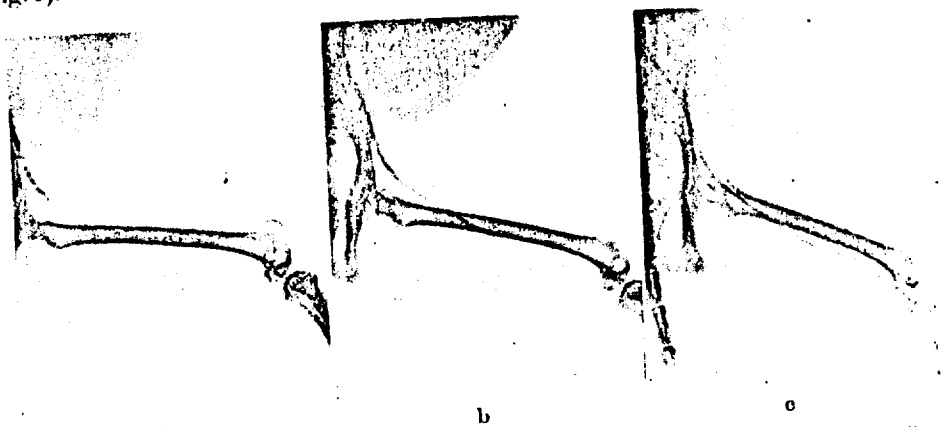
Laboratory Investigations

Fibrinolytic activity in the plasma and euglobulin fraction was determined on heated, nonheated fibrin plates (Astrup and Müllertz, 1952; Lassen, 1952). In addition, the following t were performed: Thrombelastography (Hartert, 1948), determination of plasminogen (De Vree 1965), fibrinogen (Schulz, 1955) and thrombin time (Sokal, 1955).

Results

1. In 15 animals a clot was induced by thrombin injection as previously describ Eight hours later, a saline infusion was started and continued over a period of 24 hr. Recanalization of the occluded vessel was never observed in any of the cases.

2. In 10 cats an initial dose of 750,000 u of streptokinase was administered 8 hr after clot formation and the therapy carried on with 50,000 u/hr. The clots of animals were dissolved and a complete recanalization was achieved within $2^1/_2$ hr. (Fig. 1).



a b c

Fig. 1. a) Arteriogram of the femoral artery of a cat 8 hrs after clot formation. The pointer indicate the proximal end of the clot. b) Arteriogram 2 hrs after beginning of streptokinase therapy; almos complete recanalization except for a few small parts of the clot at the site of the vessell wal c) Arteriogram $2^1/_2$ hrs after starting thrombolytic therapy; complete recanalization of the f moral artery.

3. Six cats were treated with 600 mg/kg bodyweight of sodium gluconate 8 hr after clot formation. This dose was used on the basis of that recommended by Kopp The animals received this substance dissolved in 25 ml of physiological saline over period of 30 min. None of the animals showed macroscopically or microscopically any signs of lysis of the 8 hrs old clots within 24 hrs (Fig. 2).

The laboratory investigations of the coagulatory and fibrinolytic systems did no indicate that plasmin was formed by administration of sodium gluconate (Fig. 3).

The specific tests which indicate proteolytic activity, such as, fibrin plate method euglobulin lysis time and determination of plasminogen, also remained unaltered. In addition we determined the concentration of fibrinogen and the thrombin time whic were also unchanged like those of the control animals. Blood samples were take immediately after the infusion, 15 min, 30 min, 1 hr and 3 hrs later.

4. Four more animals, in which a clot had been induced in the described manner were treated with 1200 mg/kg bodyweight. Even by this twofold increase of dose n

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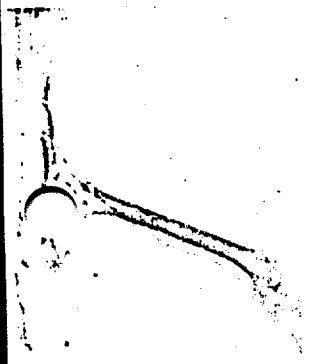

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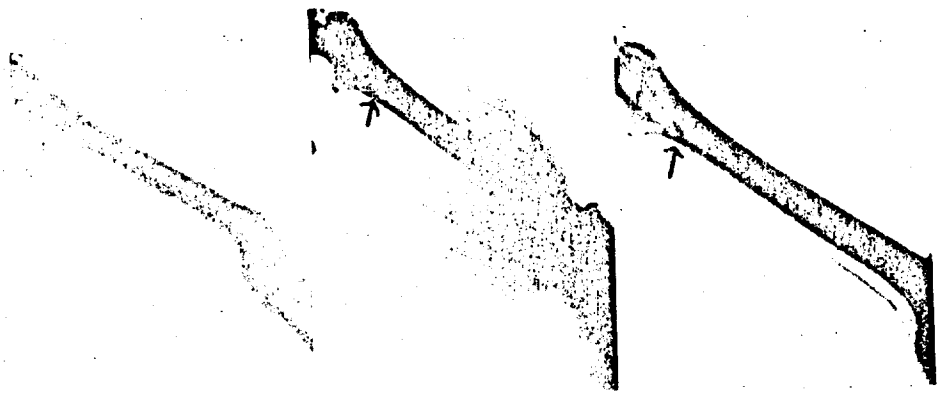

a b c

Fig. 2. a) Arteriogram of the femoral artery of a cat before inducing the clot. b) Thrombotic occlusion of the femoral artery 10 min after infusion of sodium gluconate. c) Unaltered thrombotic occlusion 12 hrs after infusion of sodium gluconate.

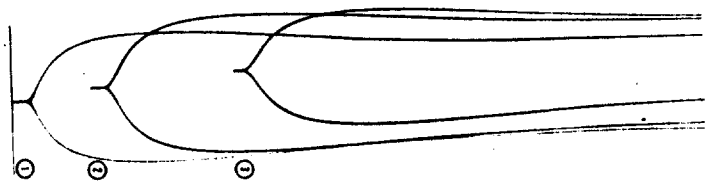

Fig. 3. Thrombelastograms of one of the cats studied: 1. 15 min after infusion of 600 mg/kg bodyweight sodium gluconate; 2. 1 hr after infusion; 3. 3 hrs after infusion.

thrombolysis was observed during 24 hrs. The fibrinolytic system also remained unchanged.

Discussion

In previous investigations we were able to show that the fibrinolytic system of the cat is comparable to that of man. The proenzyme, plasminogen, of these animals can be activated by streptokinase as well as by urokinase. Eight hour old clots induced in the femoral artery are lysed after administration of these substances, whereby, the duration of thrombolysis is dependent on the amount of streptokinase used (Hiemeyer and Rasche, 1966, 1967). The administration of nicotinic acid did not cause thrombolysis in our animal experiments, while heparin caused lysis of artificially induced clots only in exceptional cases (Rasche and Hiemeyer, 1967). The animal model of the cat offers sufficient possibilities to test the fibrinolytic and thrombolytic effect of substances to be examined.

In 1952, Kopper reported about the fibrinolytic characteristics of a filtrate of "streptococcus faecalis" which he had obtained from agar plates containing sodium gluconate. This substance exhibited, primarily, proteolytic characteristics. The plasminogen activating effect, however, was not very prominent as comparative investigations on heated and nonheated fibrin plates have shown. The same investigator observed fibrinolytic activity in extracts from human recalcified plasma clots after

addition of "streptococcus faecalis" and sodium gluconate (Kopper, 1964). In animal experiments, Kopper (1966) has tested the thrombolytic effect of fibrin extracts and sodium gluconate on artificially induced clots in the jugular vein of cats and dogs. Partial thrombolysis was observed after administration of 600 mg of sodium gluconate/kg bodyweight. Those parts of the clots which were in close contact with the endothelium of vessels exhibited an increased tendency to lysis. Under treatment, no influence on the clotting and fibrinolytic system was observed.

Tillotson and Kopper (1966) reported about the successful treatment with sodium gluconate of an aortic thrombosis in a horse. For diagnosis and indication of the therapeutic effect, only clinical criteria were used.

In further experiments, Kopper (1966) tried to clarify the thrombolytic effect of sodium gluconate. The intradermal injection of this compound mixed with fibrin extract caused a pronounced erythema with beginning hemorrhage in rats. The intradermal injection of sodium gluconate alone showed no effect, while the injection of fibrin extract alone produced an edema followed by induration. By giving fibrin extract together with 50 mg epinephrine, the rats developed a hemorrhagic necrosis at the injection site. This specific reaction has, so far, only been reported with bacterial endotoxins. The reported results might indicate that an eventual thrombolytic effect of sodium gluconate rests on its stimulation of plasminogen activators in the vessel wall at the site of the thrombus. Our experiments did not confirm a thrombolytic effect of sodium gluconate in arteries. After administration of 600 mg of this substance/kg bodyweight in 6 cats, there was no lysis within a 24 hrs treatment period of 8 hrs old clots artificially induced in the femoral artery. The same negative results were obtained after a two-fold increase of dose in an additional 4 cats. The examination of the blood coagulation and fibrinolytic systems gave no indication of a generalized plasminemia in the circulating blood of the animals.

It must be pointed out that, in contrast to Kopper who did his experiments on venous clots, we worked with clots artificially induced in arteries. From previous reports it is well known that venous thrombi in man and animals show a tendency to spontaneous lysis (Grossi, Cliffton and Cannamela, 1954; Kwaan, Lo and McFadzean, 1957), while spontaneous recanalization of thrombotic occluded arteries is seen only in exceptional cases (Hiemeyer and Zeile, 1967). This fact was explained by the reported high concentration of plasminogen activators in the endothelium of veins as opposed to the low concentration in the adventitia of arteries (Astrup et al., 1959; Todd, 1964). Further investigations are needed to prove whether sodium gluconate, in fact, induces liberation of these plasminogen activators from the wall of veins. It can be stated, however, that the substance of question has no thrombolytic effect on arterial clots in cats as was definitely shown for streptokinase and urokinase.

Summary

After Kopper (1966) reported that sodium gluconate induces thrombolysis, we tested the effect of this substance on 8 hrs old clots which had been artificially initiated in the femoral artery of cats. These clots can be lysed completely as previous experiments with streptokinase and urokinase have shown. During a 24 hrs continuous administration of sodium gluconate, in no animal a thrombolytic effect was observed. Kopper's positive results, which were obtained primarily on venous clots, lead one to believe that possibly sodium gluconate liberates plasminogen activators from the endothelium of veins, where these are in high concentrations compared to the intima of arteries.

Résumé

Après que Kopper (1966) ait indiqué que le gluconate de sodium induit une thrombolyse nous avons testé l'effet de cette substance sur des caillots âgés de 8 hrs, obtenus artificiellement dans l'artère fémorale du chat. Ces caillots peuvent être complètement lysés par la streptokinase et l'urokinase, ainsi que l'ont démontré les expériences antérieures. On n'a observé aucun effet thrombolytique chez aucun animal au cours d'une infusion continue de gluconate pendant 24 hrs. Les résultats positifs de Kopper obtenus sur des caillots veineux, font croire que le gluconate de sodium pourrait libérer les activateurs du plasminogène à partir de l'endothélium veineux, ou leur concentration est beaucoup plus élevée que dans la tunique interne des artères.

Zusammenfassung

Natriumglukonat soll nach Kopper thrombolytisch wirksam sein. Wir untersuchten die Wirkung dieser Substanz an 8 Stunden alten Standardgerinnseln, die durch Thrombininjektion in der A. femoralis der Katze hergestellt worden waren. Diese Gerinnsel können durch Streptokinase und Urokinase vollständig aufgelöst werden. Natriumglukonat ließ jedoch während einer 24stündigen Dauerinfusion keine thrombolytische oder fibrinolytische Wirkung erkennen. Es wird die Möglichkeit diskutiert, daß die von Kopper vorwiegend an venösen Thromben erhobenen Befunde auf einer verstärkten Freisetzung von Plasminogenaktivatoren aus dem Endothel der Venen stammt und beruhen.

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Bollettino della Societa Italiana di Biologia Sperimentale 14 9

The remote minimum lethal dose by intravenous application of some sodium salts Compared toxicity of some anions

By I. Simon

By the term remote minimum lethal dose by intravenous application (which others call minimum lethal dose at distance) I understand the smallest dose of a drug which, introduced into the veins at a concentration and rate not by themselves toxic, kills the animal a certain time after the end of the injection. I shall not here dwell on the great difference between immediate minimum lethal dose and remote minimum lethal dose by intravenous application, concerning which I refer the reader to my earlier publications (1). I want to mention here only that I was the first to find and to describe the curves of immediate toxicity by intravenous application (2), rediscovered and described many years later by foreign authors and rightly cleaimed for me by Beccari (3).

Having for many hears been convinced of the necessity to bring a definite order into the problem of the lethal doses, which are handled by authors today in the most personal and least precise manner imaginable (since an international unit of measurement has not yet been established), whereas they nearly always constitute the basis of our pharmacological experiments, whenever I had a chance at the Institute I determined the remote minimum lethal dose of a great variety of drugs. Through such research I acquired knowledge of a considerable number of such doses. I believe it will be useful to compile in Table I those of a number of sodium salts. The cation being always the same, the least toxic of all, we thus have a toxicity scale of many anions which is perfectly reliable, as it was prepared by uniform methods and under perfectly identical experimental conditions.

Table I

Salts		Remote minimum LD in g eq per kg	Toxicity taken as = 1 that of NaCl
Sodium sulfate	(4)	0.05650	1.13
Sodium chloride	(5)	0.05000	1
Sodium pyruvate	(6)	0.04300	0.86
Sodium bromide	(1.c.5)	0.03900	0.78
Sodium gluconate	(7)	0.03500	0.70
Sodium nitrate	(8)	0.03100	0.62
Sodium monophosphate	(9)	0.02800	0.56
Sodium acetate	(10)	0.02600	0.52
Sodium iodate	(1.c.5)	0.01400	0.28
Sodium (bi-)phosphate	(11)	0.00900	0.18
Sodium tartrate,neutral	(1.c.10)	0.00750	0.15
Sodium sulfite	(12)	0.00550	0.11
Sodium fluoride	(1.c.,5)	0.00250	0.05
Sodium persulfate	(1.c.,5)	0.00150	0.03
Sodium nitrite	(1.c.,8)	0.00050	0.01

It appears from this research that the least toxic among the tested anions is the sulfuric anion, the most toxic the nitrous, which is 100 times more toxic than the chlorine anion.

I believe that these tests and other similar ones, which I shall collect as soon as possible, can serve as a basis for an international unit of comparison.

(Translated by Carl Demrick Associates,Inc/LH/t)

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sod. glue. 9

LA DOSE MINIMA LETALE LONTANA PER VIA ENDOVENOSA DI ALCUNI SALI DI SODIO: TOSSICITA' COMPARATA DI ALCUNI ANIONI. Di I. Simon.

Con la dizione *dose minima letale lontana per via endovenosa* (che altri chiama dose minima letale *a distanza*) intendo la dose più piccola di un farmaco che, introdotta nelle vene in concentrazione e con velocità per sè non tossiche, uccide l'animale dopo un certo tempo dalla fine dell'iniezione. Non mi fermo qui a parlare della diversità profonda che passa fra dose minima letale immediata e dose minima letale lontana per via endovenosa, rimandando per questo ai lavori miei precedenti (1). Solo voglio ricordare qui che io fui il primo a trovare ed a descrivere le curve di tossicità immediata per via endovenosa (2), ritrovate poi e descritte molti anni dopo da autori stranieri e giustamente rivendicate a me da Beccari (3).

Persuaso da molti anni della necessità di dare un assetto definitivo al problema delle dosi letali, che sono negli autori quanto di più personale e di meno preciso si possa oggi immaginare (dato che non si è ancora stabilita un'unità di misura internazionale) mentre costituiscono quasi sempre la base delle nostre esperienze farmacologiche, tutte le volte che in Istituto mi si porse il destro ho fatto determinare la dose minima letale lontana di svariatissimi farmaci. A così fatte ricerche debbo la conoscenza di un numero notevole di tali dosi. Mi pare utile riportare, raccogliendole insieme, nella tabella I, quelle di numerosi sali di sodio. Essendo sempre lo stesso il catione, il meno tossico fra tutti, abbiamo così una scala di tossicità di molti anioni, perfettamente attendibile, in quanto venne fatta con metodi univoci ed in condizioni sperimentali perfettamente uguali.

Tabella I.

Sali		Dose minima letale lontana in g eq per kg	Tossicità posta = 1 quella del NaCl
Sodico solfato	(4)	0,05650	1,13
» cloruro	(5)	0,05000	1
» piruvato	(6)	0.04300	0,86
» bromuro	(1, c., 5)	0.03900	0,78
» gluconato	(7)	0.03500	0,70
» nitrato	(8)	0.03100	0,62
» monofosfato	(9)	0,02800	0,56
» acetato	(10)	0.02600	0,52
» joduro	(1, c., 5)	0.01400	0.28
» (bi) fosfato	(11)	0.00900	0.18
» tartrato neutro	(1, c., 10)	0.00750	0.15
» solfito	(12)	0.00550	0.11
» fluoruro	(1, c., 5)	0,00250	0.05
» persolfato	(1, c., 5)	0.00150	0,03
» nitrito	(1, c., 3)	0.00050	0,01

(1) *Archivio di Scienze Biologiche*, 1927, 12, 478; *Archivio it. di Sc. Farmacol.*, 1933, 2, 425.
(2) *Bollettino delle Scienze Mediche di Bologna*, 1905, 5, (ser. 8), 74.
(3) *Archives it. de Pharmac. et de Thérapie*, 1938, 58, 437.
(4) Da Val E. - *Archivio it. di Scienze Farmacol.*, 1933, 2, 445.
(5) Ravasini G. - *Ibidem*, 1933, 2, 428.
(6) Gajatto S. - (In corso di stampa).

Risulta da queste ricerche che il meno tossico fra gli anioni studiati è l'anione solforico, il più tossico il nitroso, che è 100 volte più tossico dell'anione cloro.

Credo che queste esperienze ed altre analoghe, che raccoglierò quanto prima, possano servire come base ad un'unità di confronto internazionale.

(7) Gajatto S. - (Idem).
(8) Benedet A. - *Archivio it. di Scienze Farmacol.*, 1933, 2, 461.
(9) Osser S. - *Ibidem*, 1932, 2, 478.
(10) Baratto M. - *Ibidem*, 1932, 2, 495.
(11) Gajatto S. - (In corso di stampa).
(12) Piva A. - *Archivio it. di Scienze Farmac.*, 1933, 2, 435.

(Dall'Istituto di Farmacologia della R. Università di Pisa).
Sezione di Pisa - Seduta del 23 gennaio 1939-XVII.

ANCORA DELL'AZIONE ANTIDOTICA DEL SOLFURO DI MERCURIO CONTRO L'AVVELENAMENTO MERCURIALE. Di I. Simon.

Nel 1937 comunicai alcune esperienze nelle quali dimostravo come somministrando a conigli, per via gastrica, la dose minima letale lontana di $HgCl_2$ per tale via (cc 3 di soluzione N/10 del sale per kg) e dopo 10 minuti primi, sempre *per os*, la stessa dose di Na_2S (cc 3 di soluzione N/10 per kg) gli animali si salvavano, mentre morivano se l'identico trattamento veniva praticato un'ora dopo.

Continuando le ricerche ho fatto delle esperienze assai dimostrative nelle quali potei salvare degli animali cui avevo somministrato la dose *per os*, anche dopo un'ora. Riferisco una delle esperienze assai dimostrative mentre mi riservo di riportare le altre nel lavoro *in extenso*.

Coniglio di kg 1,100.

22 giugno 1938. Alle ore 10.45 gli somministro con sonda, per via gastrica, cc 3,3 di soluzione N/10 di $HgCl_2$ diluiti in cc 20 di H_2O (g eq 0,0003 per kg), dose sicuramente letale, secondo risulta da esperienze mie e di altri). Alle ore 11.45 gli introduco, con la sonda, nello stomaco cc 6,6 di soluzione N/10 di Na_2S e ripeto lo stesso trattamento alle 18.

22 e 24 giugno. L'animale mangia poco: nell'urina emessa in piccola quantità è presente un po' d'albumina e qualche cilindro granuloso. Gli somministro nei due giorni, alle ore 9 e 18, regolarmente, per via gastrica, ogni volta cc 6,6 di soluzione N/10 di Na_2S.

L'animale si è rimesso perfettamente; l'albumina si è ridotta a traccie al 5° giorno, e poi scompare. Dopo un mese, il 23 luglio, stava benissimo e pesava g 1380.

Le esperienze continuano ed in esse vado somministrando l'antidoto a

(1) Simon I. - Questo *Bollettino*, 1937, 12, 661

Stimulation of Ovulation Processes by Means of Copper Gluconate in Heifers in Estrus (Heat)

Candidate in Biological Sciences, L. N. Gorokhov

(Presented by Academician V. K. Milovanov)

UDK (Universal Decimal Classification) 636.082.35:6 36.22/281:546.56

A. L. Paducheva (1935) and somewhat later, Fivol'd (1936) have established that following the intravenous administration of copper preparations to doe rabbits in estrus, ovulation sets in.

Harris (1941) brought about ovulation by means of the injection of a copper salt into the third ventricle of the brain. K. Vatanabe (1960) has established that copper represents a stimulent of the sexual center of the hypothalamus in so far as, in doe rabbits anesthetized with nembutal, copper preparations do not bring about ovulation. At the same time, the author tested the action, on ovulation in doe rabbits, of acetate, sulfate, ethylenediaminetetraacetate and copper gluconate, and he discovered that the last-mentioned preparation possesses the lowest toxicity and is the most effective.

V. K. Milovanov, A. P. Bereznev and L. N. Gorokhov (1965) established the possibility of making use of copper gluconate for regulating the ovulation process in females, and that of spermatogenesis in male-sires. Upon injecting the preparation in doe rabbits that had been artificially inseminated, and had not been mated (paired), there was obtained a normal litter, and in rams and bulls, copper gluconate brought about an increase in the amount and an improvement in the quality of the sperm. It was also made clear that copper gluconate preparations, even in relatively large doses, are comparatively easily endured by animals in the case of internal injections. Thus, upon administering copper gluconate to a doe rabbit, figuring on 34.2 milligrams to a kilogram of weight, no toxic phenomena were established.

We posed the problem of clarifying the possibility of employing copper

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gloconate for activating the processes of disclosing follicles in the case of artificial insemination in the head of the uterus of big horned cattle, and for enhancing their fertility.

Experiments were carried out on 208 heifers of coupling age (2 - 3 years old), with pronounced symptoms of estrus and heat. The state of the follicles in the ovaries was established according to Kedrov. There were distinguished 4 stages of fluctuation on the part of the follicles: 1) Solid (strong) (+); 2) strongly fluctuating (++); 3) Well-fluctuating (+++); and 4) Delicately fluctuating (+++++).

Copper gluconate was prepared from a medicinal preparation of calcium gluconate and chemically pure copper sulfate as a result of the reaction of double decomposition of both preparations. In 1 milliliter of copper gluconate solution there was found 9.7 milligrams of copper.

Following a careful, rectal examination, and insemination of the animals, into the jugular vein was injected a preparation of copper gluconate in varying doses: from 10 to 50 milliliters, that is, from 0.3 to 1.6 milligrams to a kilogram of animal weight. After 12 - 95 hours, or after 18 and 48 days, the heifers were salughtered, and a determination was made, in them, of the number of ovulated follicles, and also, the number of zygotes, blastocysts and embryos, and an investigation was made of the state of the sexual organs. No toxic changes were disclosed.

A study was made of the action of various doses of copper gluconate on ovulation in cows (slaughter after 24 - 96 hours). The state of the heifers, following injections of the preparation, remained satisfactory.

As may be seen from the data in Table 1, a dose of 40 - 50 milliliters of copper gluconate (1.3 - 1.6 milligrams to a kilogram of animal weight) proved to be the most effective one. The difference is statistically reliable.

Akademii Sel'skikhozyaistvennykh
NAUK, 9, 1968

Table 1

Action of Copper Gluconate on Ovulation

Dose of the Preparation	Amount of Copper to 1 kilogram of aiimal weight (in milligrams)	Amount of Cows			Difference
		Altogether	Including those with ovulation Amount	%	
10 - 30 ml	0.3 - 1.0	28	19	68 ± 9	14 ± 10
40 - 50 ml	1.3 - 1.6	51	42	82 ± 5	
Without the preparation (control)		60	37	62 ± 6	20 ± 8

Table 2

Action of 40 - 50 milliliters of Copper Gluconate on Ovulation in Connection with the State of the Follicles (Disclosure 11 - 96 hours following Investigation)

State of the Follicles	Experiment			Control		
	Total Cows	Including Those with Ovulation		Total Cows	Including Those with Ovulation	
		Number	%		Number	%
+	20	14	70 ± 10	15	7	33 ± 12
++	17	15	88 ± 8	15	7	47 ± 13
+++	17	16	94 ± 6	14	12	86 ± 8.5
++++	-	-	-	1	1	100
Total	54	45	83 ± 5	45	25	55 ± 7

In order to establish after what minimal period of time, following injection of the preparation, ovulation takes place, there were selected 7 heifers in estrus with an approximately identical degree of follicle development. Three of them were injected with an amount of 50 milliliters of the preparation, and four were left in the control (group). After 11 hours, the

heifers wer slaughtered. In all three experimental heifers, in the ovaries, an ovulation process was noted, whereas in the control, no follicles were disclosed.

The decision was made to clarify at which degree of follicle maturity the action of copper gluconate is the most pronounced on the process of ovulation (Table 2).

From Table 2 it may be seen that copper gluconate, in an optimum dose of 40 - 50 milliliters, accelerates the process of follicle disclosure independently of the degree of their maturity at the moment of injecting the preparation. The preparation is particularly effective during the first two stages of follicle development

Table 3

Action of 40 - 50 milliliters of Copper Gluconate on the Pertility of the Eggs

Group	Total Cows Examined	Eggs Lost, at the Same Time		Eggs Washed out							
				Total	Including:						
		Number	%		Zygetes		Unfertilized		Destroyed		
					Number	%	Number	%	Number	%	
Experiment	28	7	25 ± 8	21	11	53 ± 13	3	14 ± 8	7	33	
Control	15	2	13 ± 9	13	10	77 ± 14	3	20 ± 11	0	0	
Total	43	9		34	21		6		7		

A study was made of the action of copper gluconate, in doses of 15 and 40 - 50 milliliters, on the fertility of the animals. For this purpose, in animals that had been slaughtered after different periods following insemination, from the oviducts were washed out egg cells, or else, from the uterus were extracted blastocysts or embryos.

The experiment, carried out on 70 heifers, indicated that 15 milliliters of copper gluconate did not indicate any positive effect on the fertility. In Table 3 are set forth data with respect to the action of 50 milliliters of

copper gluconate on the fertility of the eggs.

The data in Table 3 attest to the fact that in a group of cows, which had been subjected to an injection of copper gluconate, the number of fertilized eggs is considerably lower than in the control (53% as against 77%). At the same time, it is not difficult to observe that a reduction in the number of zygotes, in the experiment, took place, for the main part, as a result of increasing the number of destroyed eggs (33% as against 0), and eggs lost (25% as against 13).

It was assumed that a greater number of destroyed and lost eggs, in the experimental group, is associated with the acceleration of ovulation under the influence of copper gluconate.

In order to clarify this problem, we made a study of the dependence of the results of the fertility on the state of the follicles at the moment of insemination and injection of copper gluconate (Table 4).

As may be seen from the data in Table 4, in the experimental group, the fertility of the eggs grows regularly together with an increase in the degree of maturity of the follicle from 0 - 37% to 89%. At the same time, there is observed a reduction in the number of destroyed (from 75 to 38%) and lost (from 33 to 18%) eggs. In the control group of heifers, the fertility of the eggs likewise grows noticeably -- up to 86% in the third stage of development of the follicle. However, in contrast with the experimental group, at this point there are absolutely no destroyed eggs cells, and 25% of lost eggs are observed only in the group of heifers with well-fluctuating follicles.

The fact that there is found a large number of destroyed egg cells in animals with solid follicles in the ovaries, and the absence of such egg cells in the control, yields a basis for the assumption that the injection of copper gluconate leads to an acceleration in the process of ovulation even of not entirely mature follicles, and to the emergence of immature eggs into the

oviducts where they are destroyed. Premature ovulation, evidently, likewise brings about a considerable percent (33%) of egg losses in experimental heifers, with the follicles in the first stage of maturity.

In this manner, the experiments have shown that a reduced fertility of the eggs is observed only in the case of strong (solid) and strongly fluctuating follicles. In cows with well-fluctuating follicles, the fertility of the eggs corresponded to the control and attained 89%. This makes it possible to conclude that the application of copper gluconate, with the purpose of accelerating ovulation, may yield an effect merely during the third stage of development of the follicles, and it is practically ineffective in all other cases.

Table 4

Action of 40 - 50 milliliters of Copper Gluconate on the Fertility of Eggs in Connection with the State of the Follicles in the Ovary of Cows (Disclosure after 48 - 96 hours)

State of Follicles	Experiment: Total Cows	Eggs Lost	Eggs Washed Out: Total	Including: zygotes	unfertilized	destroyed
+ number	6	2	4	0	1	3
%	100	33	100	0	25	75-25
++ number	11	3	8	3	2	3
%	100	27	100	37± 22	25	38± 17
+++ number	11	2	9	8	-	1
%	100	18	100	89± 11	0	11 ± 10
	Control					
	1	1	1	0	1	0
	100	0	100	0	100	0
	5					
	100	-	5	4	1	0
	9	0	100	80±20	20±20	0
	100	2	7	6	1	0
		25	100	86±14	14±14	0

Conclusions

1. Optimum doses of copper gluconate, equal to 40 - 50 milliliters, using intravenous injections, accelerate the process of ovulation in heifers of coupling (mating) age, in the state of estrus, in all stages of development of follicles in the overies.

2. In the presence, in the ovaries, of immature follicles, copper gluconate brings about a premature ovulation, and an emergence of incomplete egg cells. This brings about a low fertility on the part of the eggs, whereas against a background of mature follicles, the fertility of the eggs corresponds to the control.

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СТИМУЛЯЦИЯ ПРОЦЕССОВ ОВУЛЯЦИИ ГЛЮКОНАТОМ МЕДИ У ТЕЛОК В ОХОТЕ

(Представлено академиком В. К. Милованом)

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А. Л. Падучева (1935) и несколько позже Фивольд (1936) установили, что после внутривенного введения препаратов меди у крольчих в охоте наступает овуляция.

Харрис (1941) вызывал овуляцию путем инъекции соли меди в третий желудочек мозга. К. Взятнабе (1960) установил, что медь является стимулятором полового центра гипоталамуса, поскольку у внестезиризованных кембуталом крольчих препараты меди не вызывают овуляции. Одновременно автор испытал действие на овуляцию у крольчих ацетата, сульфата, этилендиаминтетрацетата и глюконата меди и нашел, что последний препарат обладает наименьшей токсичностью и является наиболее эффективным.

В. К. Милованов, А. П. Березнев и Л. Н. Горохов (1965) установили возможность применения глюконата меди для регуляции процесса овуляции у самок и сперматогенеза у самцов-производителей. При инъекции препарата искусственно осемененным крольчихам, не спаренным с самцом, был получен нормальный приплод, а у баранов и быков глюконат меди вызывал увеличение количества и улучшение качества семени. Было также выяснено, что препараты глюконата меди даже в относительно больших дозах животные сравнительно легко переносят при внутривенных инъекциях. Так, при введении крольчихе глюконата меди из расчета 34,2 мг на килограмм веса не было установлено токсических явлений.

Мы поставили задачу выяснить возможность применения глюконата меди для активизации процессов вскрытия фолликулов при искусственном осеменении маточного поголовья крупного рогатого скота и повышения его оплодотворяемости.

Опыты проводили на 208 телках случного возраста (2—3 г) с выраженными признаками охоты и течки. Состояние фолликулов в яичниках устанавливали по Кедрову. Различали 4 стадии флюктуации фолликулов: 1) плотный (+), 2) плотно флюктуирующий (++), 3) хорошо флюктуирующий (+++) и 4) нежно флюктуирующий (++++).

Глюконат меди готовили из медицинского препарата глюконата кальция и химически чистой сернокислой меди посредством реакции обменного разложения обоих препаратов. В 1 мл раствора глюконата меди содержалось 9,7 мг меди.

После тщательного ректального исследования и осеменения животных в яремную вену инъецировали препарат глюконата меди в разных дозах: от 10 до 50 мл, то есть от 0,3 до 1,6 мг на килограмм веса животного. Через 12—96 час. или через 18 и 48 дней телок убивали, определяли у них число овулировавших фолликулов, а также количество зигот, бластоцист и эмбрионов и исследовали состояние половых органов. Токсических изменений обнаружено не было.

Было изучено действие разных доз глюконата меди на овуляцию у коров (убой через 24—96 час.). Состояние телок после инъекций препарата оставалось удовлетворительным (табл. 1).

Таблица 1

Действие глюконата меди на овуляцию

Доза препарата	Количество меди на 1 кг веса животного (мг)	Количество коров			Разница
		всего	в том числе с овуляцией		
			количество	%	
10—30 мл	0,3—1,0	28	19	68±9	14±10
40—50 мл	1,3—1,6	51	42	82±5	
Без препарата (контроль)		60	37	62±6	20±8

Как видно из данных таблицы 1, доза в 40—50 мл глюконата меди (1,3—1,6 мг на килограмм веса животного) оказалась наиболее эффективной. Разница статистически достоверна.

Для того чтобы установить, через какой минимальный срок после инъекции препарата происходит овуляция, было отобрано 7 телок в охоте с примерно одинаковой степенью развития фолликулов. Трем из них было инъецировано по 50 мл препарата, а четыре оставлены в контроле. Через 11 час. телки были убиты. У всех трех опытных телок в яичниках отмечен процесс овуляции, тогда как в контроле не было вскрытых фолликулов.

Было решено выяснить, при какой степени зрелости фолликула наиболее выражено действие глюконата меди на процесс овуляции (табл. 2).

Из таблицы 2 видно, что глюконат меди в оптимальной дозе 40—50 мл ускоряет процесс вскрытия фолликулов независимо от степени зрелости их в момент инъекции препарата. Особенно эффективен препарат при первых двух стадиях развития фолликулов.

Таблица 2

Действие 40—50 мл глюконата меди на овуляцию в связи с состоянием фолликулов (вскрытие через 11—96 час. после исследования)

Состояние фолликулов	Опыт			Контроль		
	всего коров	в том числе с овуляцией		всего коров	в том числе с овуляцией	
		количество	%		количество	%
+	20	14	70±10	15	5	33±12
++	17	15	88±8	15	7	47±13
+++	17	16	94±6	14	12	86±8,5
++++	—	—	—	1	1	100
Всего	54	45	83±5	45	25	55±7

Таблица 3

Действие 40—50 мл глюконата меди на оплодотворяемость яиц

Группа	Всего исследовано коров	При этом потерянно яиц		Вымыто яиц							
		число	%	всего	в том числе:						
					зигот		неоплодотворенных		разрушенных		
					число	%	число	%	число	%	
Опыт	28	7	25±8	21	11	53±13	3	14±8	7	33	
Контроль	15	2	13±9	13	10	77±14	3	20±11	0	0	
Всего	43	9		34	21		6		7		

Было изучено действие глюконата меди в дозах 15 и 40—50 мл на оплодотворяемость животных. Для этого у убитых через разные сроки после осеменения животных из яйцеводов вымывали яйцеклетки либо из матки извлекали бластоцисты или эмбрионы.

Опыт, проведенный на 70 телках, показал, что 15 мл глюконата меди не оказало положительного влияния на оплодотворяемость. В таблице 3 приведены данные по действию 50 мл глюконата меди на оплодотворяемость яиц.

Данные таблицы 3 свидетельствуют о том, что в группе коров, подвергнутых инъекции глюконата меди, число оплодотворенных яиц значительно меньше, чем в контроле (53% против 77%). При этом нетрудно заметить, что снижение числа

Таблица 4

Действие 40—50 мл глюконата меди на оплодотворяемость яиц в связи с состоянием фолликулов в яичнике коров (вскрытие через 48—96 час.)

Состояние фолликулов	Опыт						Контроль				
	всего коров	потерянно яиц	вымыто яиц				всего коров	потерянно яиц	вымыто яиц		
			всего	в том числе:					всего	в том числе	
				зигот	неоплодотворенных	разрушенных				зигот	неоплодотворенных
+ число	6	2	4	0	1	3	1	0	1	0	1
%	100	33	100	0	25	75±25	100	0	100	0	100
++ число	11	3	8	3	2	3	5	1	5	4	1
%	100	27	100	37±22	25	38±17	100	0	100	80±20	20±20
+++ число	11	2	9	8	1	1	9	2	7	6	1
%	100	18	100	89±11	0	11±10	100	25	100	86±14	14±14

зигот в опыте произошло главным образом за счет увеличения количества разрушенных яиц (33% против 0) и потерянных яиц (25% против 13%).

Было предположено, что большее число разрушенных и потерянных яиц в опытной группе связано с ускорением овуляции под действием глюконата меди.

Для выяснения этого вопроса мы изучили зависимость результатов оплодотворяемости от состояния фолликулов в момент осеменения и инъекции глюконата меди (табл. 4).

Как видно из данных таблицы 4, в опытной группе оплодотворяемость яиц закономерно возрастает вместе с увеличением степени зрелости фолликула с 0—37% до 89%. Одновременно наблюдается снижение числа разрушенных (с 75 до 38%) и потерянных (с 33 до 18%) яиц. В контрольной группе телок оплодотворяемость яиц тоже заметно возрастает — до 86% при третьей стадии развития фолликула. Но в отличие от опытной группы здесь совершенно нет разрушенных яйцеклеток, а 25% потерянных яиц наблюдается только в группе телок с хорошо флюктуирующими фолликулами.

Факт нахождения большого числа разрушенных яйцеклеток у животных с плотными фолликулами в яичниках и отсутствие таких яйцеклеток в контроле дают основание предположить, что инъекция глюконата меди приводит к ускорению процесса овуляции даже не совсем зрелых фолликулов и выходу незрелых яиц в яйцеводы, где они разрушаются. Преждевременной овуляцией, очевидно, вызван также значительный процент (33%) потери яиц у опытных телок с фолликулами в первой стадии зрелости.

Таким образом, опыты показали, что пониженная оплодотворяемость яиц наблюдается только при плотном и плотно флюктуирующем фолликулах. У коров с хорошо флюктуирующими фолликулами оплодотворяемость яиц соответствовала контролю и достигала 89%. Это дает возможность заключить, что применение глюконата меди с целью ускорения овуляции может дать эффект лишь при третьей стадии развития фолликулов и практически бесполезно во всех других случаях.

ВЫВОДЫ.

1. Оптимальные дозы глюконата меди, равные 40—50 мл, при внутривенных инъекциях ускоряют процесс овуляции у телок случного возраста в состоянии охоты при всех стадиях развития фолликулов в яичниках.

2. При наличии в яичниках незрелых фолликулов глюконат меди вызывает преждевременную овуляцию и выход неполноценных яйцеклеток. Это приводит к низкой оплодотворяемости яиц, тогда как на фоне зрелых фолликулов оплодотворяемость яиц соответствует контролю.

Всесоюзный научно-исследовательский институт животноводства

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ИНТЕРЬЕРНЫЕ ОСОБЕННОСТИ ПО КРАСНОЙ КРОВИ СУФФОЛЬКСКИХ, ЦИГАЙСКИХ И СУФФОЛЬК-ЦИГАЙСКИХ ЯРОК

(Представлено академиком А. И. Николаевым)

УДК 636.32/.38:611.018.5

Нами проведен опыт скрещивания импортных баранов суффолькской мясо-шерстной породы с цигайскими ярками. Предметом исследования интерьера была красная кровь. Было отобрано по принципу аналогов по пять ярок суффолькской, цигайской пород и суффольк-цигайских помесей первого и второго поколения. Все отобранные ярки имели крепкую конституцию.

Кровь для исследования брали из яремной вены в 1-месячном, 4-месячном и 6-месячном возрасте. В крови определяли процентное содержание гемоглобина по Сали, количество эритроцитов в 1 мм³ крови.

В месячном возрасте кровь ягнят различных породностей по количеству эритроцитов в 1 мм³ практически не имела различий (табл. 1). Самая большая разница по этому показателю составляла 0,58 млн/мм³ между ярками цигайской породы и помесями второго поколения, но и эта разница была статистически недостоверной.

У одномесячных ягнят самое низкое содержание гемоглобина оказалось у ярок цигайской породы, затем у помесей первого поколения, у суффолькских и помесей второго поколения. Статистически достоверной была разница между сверстницами исходных пород, цигайскими и помесями второго поколения.

В 4-месячном возрасте наибольшее ко-

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The Safety and Fate of Potassium Sodium Copper Chlorophyllin and Other Copper Compounds*

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The growth rate, survival, blood and urine factors, and ability to conceive, were normal in albino rats, who were fed up to three percent of potassium sodium copper chlorophyllin in their diets over their life span. There was no other indication of toxicity. No gross or microscopic pathology could be attributed to this diet. No metal toxicity was evident and photosensitization did not occur.

WILLSTÄTTER and Fisher, in 1915 and 1930, when they published their climatic researches on chlorophyll, stimulated the interest of Emil Bürgi, a Swiss (1), who in 1916 advocated the use of chlorophyll and its derivatives as therapeutic agents. Bürgi's enthusiasm was predicated upon the structural resemblance between erythrocyte hemin and the green pigment from the chloroplasts as disclosed by Willstätter and Fisher. However, in this suggestion of therapeutic value, Bürgi's thought that chlorophyll might be a hemopoetic agent was not original, for Verdeil (2) had suggested this use almost a century before. Furthermore, even before Willstätter and Fisher, the relationship between hemin and chlorophyll was suspected by Hoppe-Seyler (3), who had separated a red porphyrin from chlorophyll which was similar in many respects to one obtainable from hemin. Nevertheless, interest in chlorophyll or its derivatives as therapeutic agents lagged until the enthusiasm of Bürgi launched numerous scientific investigations which followed the marketing by his associates of chlorophyll products: both as chlorophyll itself, and as water-soluble derivatives of chlorophyll. The usefulness of these was a controversial issue almost from the start; careful investigators both supported and denied the suggested values. Lately chlorophyll, and especially the water-soluble derivatives of chlorophyll have become increasingly of interest, especially to stimulate tissue healing (4), and as deodorants, though their original suggested use as hemopoetic agents no longer persist.

Bürgi, Gordonoff, Grigoriew, Patek (5), and others, who were the initiators, employed "pure" chlorophyll, as well as various chlorophyll fractions in their studies, terming the products: sodium chlorophyllin, sodium and potassium chlorophyllin, "Nachlorophyllate," pheophytin and chlorin. That none of these were likely pure products, can be conceded in view of the difficulties presently encountered in preparing pure materials of this nature. The names used by Bürgi and others to describe the products employed were no doubt loosely applied. Actually little concern seems to have been given to their true identity, probably because it was impractical to prepare pure products, and even impractical to ascertain the identity of the fractions in the available marketed materials. This situation continues today, for we find that the extensively used metallo-complex known commercially as potassium sodium copper chlorophyllin, is in reality a mixture, and the term will be used with that meaning in mind in this paper. Examination of a typical production lot of this complex by Pickel, Scanlan, and Heggie (6) is illustrative of the complexity of the commercially marketed product (Table I). This is apparently typical of the acceptable and generally marketed, potassium sodium copper chlorophyllin.

TABLE I.—ANALYSIS OF LOT 89

	Per cent
Ash	32.33
Nitrogen (Kjeldahl)	3.88
Sodium	0.16
Potassium	14.49
Copper (total)	4.10
Copper (ionic at *p*H 3)	0.24
Phytol	None
Yellow Fraction	0.2
Isochlorin e_4	12.
Chlorin e_6[a]	36.
Assay (N. N. R.)	78.5
*p*H 1% solution	9.7

[a] Now known to be a mixture of chlorin e_6 and rhodin g_7.

Bürgi and others, as a result of their investigations in regard to therapeutic usefulness, published a considerable amount of data on various aspects of chlorophyll and its degradation pro-

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cts. Some observations as to safety or toxicity naturally followed, but in these studies the therapeutic doses employed did not lend themselves to the more drastic approach which is followed today to establish safety or a tolerance level. A conclusion of safety within the realm of the necessities of the time was largely *a priori* drawn, likely by reason of chlorophyll's natural origin and on the basis of man's constant ingestion of chlorophyll in his diet. This conclusion was supported by a limited amount of animal experimentation at a toxic level. With the resurgence of interest in chlorophyll about 1940, especially regarding the water-soluble fractions and particularly the copper complex, Smith (7) investigated the probable safety of this metallic complex derivative of saponified and substituted chlorophyll, which he described as "sodium copper chlorophyllin." Employing 0.2 and 2 per cent aqueous solutions, Smith administered the material orally, intravenously, and intraperitoneally, for periods up to five days, to rabbits, and up to eight days to humans. He observed no ill effects in any of the subjects. However, these administrations were over a short period of time and do not furnish an answer relative to its chronic toxicity. Though Smith concluded from his experimental work, that there were no toxic effects, Corwin (8) postulated that the copper of this chlorphyll derivative might be available, thereby capable of causing liver damage, if ingested in sufficient quantity. Furthermore, Corwin concluded that a high intake of any chlorophyll derivative, especially those of simple form, might be inadvisable and extremely dangerous if effective amounts reached the blood stream. He concluded however, that such absorption is apparently not likely inasmuch as there is no evidence of such an effect among the population at large.

Zirm and Kilches (9), using what they describe as a water-soluble C^{14} labeled sodium magnesium chlorophyllin, found that it was not transported across the gastrointestinal membranes. There was no indication of its presence in the blood or of it being deposited in the tissues. White mice were employed and 10 mg. was administered orally. Excretion in the feces persisted for eight days, after the single dose.

An extensive review of the literature on chlorophyll has been made by Eddy (10). Reference to this will furnish additional information upon the history and use of these materials.

The present internal human uses of potassium sodium copper chlorophyllin are as a breath deodorant, which involves the ingestion of approximately 4 mg. per lozenge, and as a systemic deodorant, employing tablets containing 10 mg. As the compound contains not more than 6 per cent of copper, these doses would contribute a maximum of 0.24 mg. and 0.60 mg. respectively, of total copper, of which 0.012 mg. and 0.045 mg. is in ionic form at a *p*H of 3.

There is no general unanimity of opinion that the population at large requires a source of copper supplementary to the average diet, which is estimated by Darby (11) to contain 2–4 mg. per day, though a daily intake of 2 mg. is viewed as being the minimum required. That copper is a necessary element in the daily human diet is accepted, though the role it plays in nutrition is not understood (12). However, in animals, experimental anemia may be developed even in the presence of an adequate iron intake when copper is absent (13). Many common foods furnish rather considerable amounts of copper (14), and in some areas the copper intake in foods is increased by the practice of enhancing the color of canned green vegetables by "coppering" or by employing brass vessels for the purpose of cooking. Drummond (15) concluded that 50–150 mg. of copper per Kg. of food, may be present without harm; Long (16), that it is nontoxic when combined with the chlorophyll of green vegetables. Waltner (17) that 0.1 per cent in the diet has no effect upon rats; Huber (18) that 10 mg. Kg. daily to guinea pigs for seventy two days has no effect; Kiyooka (19) that 70 mg. daily to man is without effect. The intake of copper by humans may, therefore, in most instances be considerable and the amount that may be ingested by reason of the use of potassium sodium copper chlorophyllin may play no significant part. Especially may this be true if only the ionizable copper is available to the organism.

However, toxic effects follow a high intake of copper, over prolonged periods: Waltner states that 2 per cent in the diet of rats results in death in four to five days. Kiyooka that 750 mg. daily to man is toxic. Individual large doses are not likely to be harmful if the subject is physiologically capable of emesis, for such large doses are irritating to the stomach and vomiting follows their ingestion.

The likelihood that copper will exhibit a toxic effect when it is continuously ingested is therefore governed by the level of intake and the availability of the ingested copper for transfer across the gastrointestinal membrane. However, administration of copper over a prolonged period, even at a low concentration, if it is available for absorption, results in increased copper storage especially in the liver and spleen (20).

The suggestion by Corwin that a high intake of

chlorophyll derivatives might result in photosensitization by reason of circulating porphyrins is also of interest. Chlorophyll itself, when freshly released, by direct contact with plants upon areas of the skin not normally exposed to light, is alleged to result in photosensitization. Furthermore, photosensitization is known to follow the administration of hemoglobin derivatives (hematoporphyrin), and some dyes, which effects have been employed as therapeutic measures. Photosensitization is not uncommon, as contact with parsnip, bergamot oil, or some fluorescent dyes (lip stick) may also result in contact photosensitization. Furthermore, cud chewers (ruminants) develop phylloerythrin, presumably from chlorophyll, in their digestive tract, a porphyrin known to be photosensitizing. This is normally excreted in the bile, but if excretion is prevented, photosensitization may follow. Indeed phylloerythrin is found in human feces and an increase in the amount present appears to follow an increase of chlorophyll intake. Furthermore, chlorophyll urinary porphyrin appears to be isomeric with blood porphyrin. Rothemund in his extensive review on chlorophyll outlines the possibilities of photosensitization (21).

Safety studies on potassium sodium copper chlorophyllin, employing the long-term study techniques designed in the manner of present-day practice, do not appear in the literature. The lack of this information and the presently important aspect of water-soluble chlorophyll products, resulted in the initiation of studies employing mice, rats, and guinea pigs as test animals. Because of the thought that the copper content of "chlorophyllin" might induce a toxic response, studies were also initiated using copper sulfate and copper gluconate as the copper salts for comparison of effects. The copper of these copper salts is 100 per cent ionizable, whereas the copper of potassium sodium copper chlorophyllin is about 5 per cent ionizable, fully 95 per cent being bound in a chelate position.

In designing the studies, consideration was given to many viewpoints, i. e., will these modified chlorophylls, when consumed at high level, influence growth, mating, or the normality of the tissues; will this "chlorophyllin" induce a high porphyrin content of the blood resulting in the possibility of photosensitization; will the content of 4 to 5 per cent of total copper in the "chlorophyllin" be toxic; will the copper interfere with the availability and use of vitamin C, which is known to deteriorate rapidly in the presence of copper.

The material commercially available is termed potassium sodium copper chlorophyllin, and it will be referred to as such, though it is now recognized that pure chlorophyllins do not comprise a major part of the commercial product; and for brevity in some instances the term "chlorophyllin" in quotation marks will be employed. In marketing it is also described as a "water-soluble chlorophyll" though there are many such soluble derivatives of chlorophyll, and the potassium sodium copper chlorophyllin complex is probably a colloid in polyelectrolyte dispersion (22).

ACUTE TOXICITY OF POTASSIUM SODIUM COPPER CHLOROPHYLLIN

In the acute toxicity tests Swiss mice, Taconic Farms[1] strain, and Sprague-Dawley[2] rats were used. The mice weighed between 18 and 21 Gm., the rats 180–240 Gm. To the mice the chlorophyllin was administered orally, in 15% aqueous solution by stomach tube and by intraperitoneal route. The rats received a diet containing 15% of the "chlorophyllin."

The LD_{50}, oral, for male mice was found to be 7.0 Gm. per Kg.; the approximate LD_0 being 5.0 Gm. per Kg. and the LD_{100}, 12 Gm. per Kg. Observations were made over a period of seven days, but no change in the survival rate occurred after seventy two hours. The LD_{50}, intraperitoneal, for mice was 0.19 Gm. per Kg.; the approximate LD_0 being 0.13 Gm. and the LD_{100} 0.32 Gm. All solutions were adjusted to a *p*H of 7.6 before administering, by the addition of the hydrochloric acid. When water-soluble chlorophyll is administered by the intraperitoneal or intravenous routes, its high tinctorial power and colloidal nature results in all the tissues being tinted green and concentration in the RES. This coloration is noticeable in the ears and eyes of albino animals.

Five male and four female young adult rats were fed a diet comprising 15% potassium sodium copper chlorophyllin and 85% Rockland rat meal. The animals were maintained in individual cages for a period of ten days, during which no deaths occurred. The males ingested 10.2 Gm. of the "chlorophyllin," and the females who consumed more food, had an intake of 13.4 Gm. Some loss in weight occurred in both sexes, a greater loss among the males, due to refusal of the food mixture. The over-all intake during the ten days averaged above 50 Gm. "chlorophyllin" per Kg. of rat. During a ten-day period the LD_0 for rats was therefore above 50 Gm. per Kg.

LIFE CYCLE TOXICITY STUDY (CHRONIC TOXICITY) ON RATS OF POTASSIUM SODIUM COPPER CHLOROPHYLLIN

Weanling rats of the Sprague-Dawley strain were housed individually to prevent coprophagy and in a manner whereby an accurate record of food and water intake of each animal would be assured. Two hundred animals were included in this study, forty in each of the feeding levels, namely 0.1%, 0.5% and 3% of the "chlorophyllin" in the diet and a

[1] Taconic Farms, Inc. Germantown, N. Y.
[2] Sprague-Dawley Company, Madison, Wis

group upon the basic diet alone, which was Rockland rat diet in meal form, an adequate diet for rats consisting of required food elements.

In order to hold a reasonably consistent ratio of "chlorophyllin" intake per gram of animal weight over the wide life cycle weight range of the test animals, a moving percentage in the diet was maintained. During the first fourteen days on test when food intake is highest per gram of animal weight, 25% of the stated concentrations were fed; during the second fourteen days, 50% of the stated concentrations were fed; thereafter for the balance of the study the 0.1, 1.0, and 3.0% concentration in the diets were maintained.

The weight of each animal was determined weekly as well as the amount of food and water consumed. Animals were individually inspected no less than three times each week. Thus, there were four groups of animals, each group comprised twenty males and twenty females, and in each group a littermate of the same sex.

Growth (Gain in Weight).—During the first twenty months, the animals were on test, male animals were receiving up to 3% of "chlorophyllin" in the diet (roughly 3 Gm. "chlorophyllin" per Kg. of animal) and increased steadily and constantly in weight. An average gain in weight of 470 Gm. occurred among the control animals, and of 494 Gm. among the animals on the diet containing 3% "chlorophyllin" (Fig. 1). Female animals likewise gained in weight constantly. An average gain of 278 Gm. occurred among the control animals and 255 Gm. among those receiving 3% of the test material in the diet (Fig. 2). After this period of twenty months, terminal sacrifice, death due to age, and emaciation due to old age affected all groups so that the average total body weight and gain in weight show greater fluctuations.

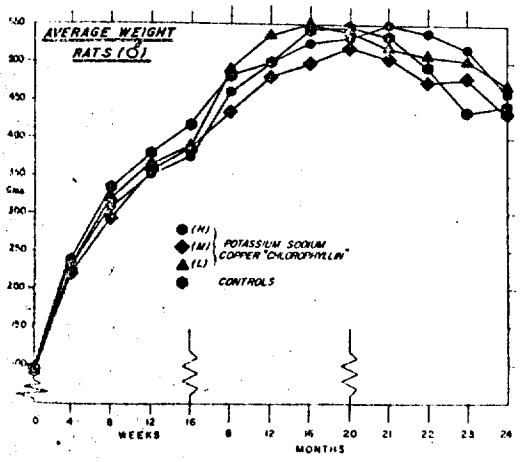

Fig. 1

The growth of animals on the other levels of intake, i. e., 0.1% and 1.0% was comparable. The gain in weight of the several groups during their growth periods as compared with control animals was not significantly different. The increase in weight, the standard error of the average weights and the number of animals in each group at specified

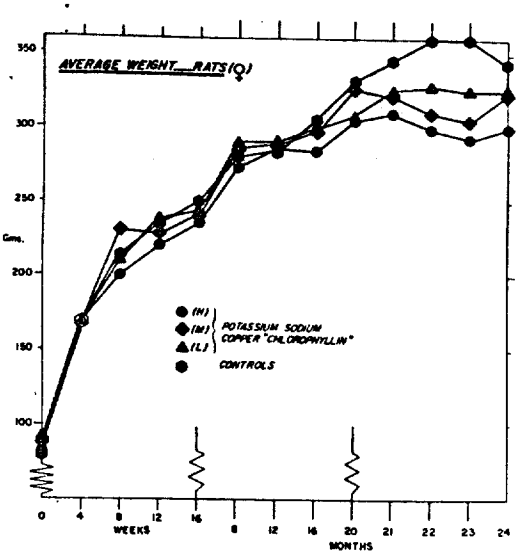

Fig. 2

periods during the study show no significant differences between the groups on the several levels of "chlorophyllin" and the control group, within the sexes (Table II). As the study neared termination, after the ninety-ninth week, the variations in weight within groups was larger as evidenced by the greater standard error (Table II), emaciation of old age, especially among the males, resulted in greater weight variations, and individual loss of weight.

Food Usage and Material Intake.—The net food intake over a ninety-three week period averaged within 3% for all groups, an average net daily food use of approximately 20 Gm. for males and 16 Gm. for females. During this period the females on the high (3%) intake of "chlorophyllin" consumed an amount greater than their own body weight (Table III). Male animals on the average consumed an amount equal to about four fifths their body weight.

The efficiency of food use expressed as grams of weight gain per gram of food consumed, was calculated for the periods terminating at the eighth and twelfth weeks. This usage did not differ materially between those animals on the control diet and those receiving "chlorophyllin" in their diets (Table IV).

Mating.—Five males and five females from each of the feeding levels were mated. The respective male of a specified level was mated with a female of the same level, allowing a one week residence of the male. Not all pairs conceived. The control animals whelped an average of 7.2 pups; the test groups 6.5-9 pups. From the control pups an average of 5.2 were raised to maturity; from the test groups, 4.5-6.2 pups.

Mortality.—The survival of animals was satisfactory: 30% being lost from the control group, 22% and 18% from the 3% and 0.1% diet groups, respectively (Fig. 3), the majority of deaths occurring during the last few weeks.

Blood and Urine Examination.—Routine hematologic and urine examinations were made several times during the twenty months, as well as nonprotein nitrogen determinations upon the blood. The factors determined were within normal limits.

TABLE II.—AVERAGE BODY WEIGHT OF RATS RECEIVING POTASSIUM SODIUM COPPER CHLOROPHYLLIN IN DIET

		0 week	4 weeks	8 weeks	12 weeks[b]	6 months	1 year	99 weeks[c]
Females								
Controls		52 ± 3.0 Gm.	168 ± 3.5 Gm.	212 ± 3.6 Gm.	235 ± 3.5 Gm.	261 ± 4.2 Gm.	286 ± 5.8 Gm.	359 ± 33.6 Gm.
	N	20	20	20	16	15	15	11
Potassium sodium copper chlorophyllin								
0.1% in diet		52 ± 1.6[a]	168 ± 3.4	210 ± 9.3	237 ± 4.6	267 ± 4.7	288 ± 4.8	328 ± 13.4
	N	20	20	20	15	15	15	11
1.0% in diet		50 ± 2.4	167 ± 3.8	229 ± 5.1	227 ± 3.7	258 ± 4.5	290 ± 4.2	308 ± 43.4
	N	22	22	22	18	18	18	11
3.0% in diet		49 ± 3.0	165 ± 3.3	202 ± 3.6	220 ± 4.1	248 ± 4.7	287 ± 8.0	299 ± 28.9
	N	20	20	20	16	16	16	9
Males								
Controls		53 ± 3.4	223 ± 21.0	335 ± 4.6	382 ± 27.2	422 ± 24.1	500 ± 15.4	440 ± 92.1
	N	20	20	20	16	15	15	8
Potassium sodium copper chlorophyllin								
0.1% in diet		54 ± 3.6	228 ± 21.1	320 ± 20.9	366 ± 5.8	421 ± 6.3	533 ± 10.0	519 ± 60.6
	N	20	20	20	16	16	16	8
1.0% in diet		54 ± 2.6	227 ± 5.5	294 ± 7.3	360 ± 5.6	403 ± 10.8	481 ± 15.9	461 ± 8.8
	N	18	18	18	14	14	14	7
3.0% in diet		54 ± 3.7	223 ± 7.4	311 ± 6.0	352 ± 8.0	393 ± 10.0	500 ± 15.8	495 ± 15.5
	N	20	20	20	16	16	16	7

[a] Standard Error = $\sqrt{\Sigma d^2/N(N-1)}$.
[b] Depleted by autopsy at 10th week.
[c] Depleted by autopsy at 52nd week.

TABLE III.—AVERAGE AMOUNT OF FOOD AND OF "CHLOROPHYLLIN" IN GRAMS CONSUMED PER RAT IN 93 WEEKS

Chlorophyllin in Diet	Food Consumed		"Chlorophyllin" Consumed	
	Females	Males	Females	Males
Control	10,760	12,810	None	None
Chlorophyllin				
0.1%	10,730	13,110	10.6	12.5
1.0%	10,680	13,050	105.5	129.1
2.0%	11,240	13,610	333.2	403.9

TABLE IV.—FOOD EFFICIENCY AND GAIN IN WEIGHT

	Gain in Weight, Gm. 8 weeks	12 weeks	26 weeks	52 weeks[a]	Food Efficiency, Gm. Gained per Gm. Food Consumed 8 weeks	12 weeks
Males						
Controls	238	285	326	404	0.23	0.17
Potassium sodium copper chlorophyllin						
0.1% in diet	224	270	325	437	0.23	0.18
1.0% in diet	202	268	310	388	0.21	0.17
3.0% in diet	218	260	300	407	0.20	0.15
Controls	229	301	359	378	0.24	0.20
Copper sulfate						
(530 p. p. m. Cu)	202	286	353	359	0.25	0.22
(1,600 p. p. m. Cu)	176	209	211	264	0.23	0.17
Copper gluconate (1,600 p. p. m. Cu)	197	252	193	144	0.24	0.19
Females						
Controls	129	152	178	204	0.15	0.12
Potassium sodium copper chlorophyllin						
0.1% in diet	119	146	176	197	0.15	0.12
1.0% in diet	139	137	168	200	0.16	0.11
3.0% in diet	127	137	164	204	0.15	0.10
Controls	131	153	188	192	0.15	0.12
Copper sulfate						
(530 p. p. m. Cu)	140	165	204	194	0.19	0.14
(1,600 p. p. m. Cu)	125	151	147	184	0.17	0.13
Copper gluconate (1,600 p. p. m. Cu)	125	160	129	107	0.16	0.13

[a] For copper sulfate and copper gluconate, 35 weeks.

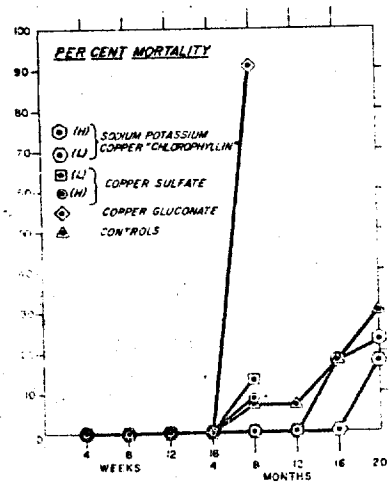

Fig. 3

Oxygen Carrying Capacity.—Hemoglobin was also determined by the Van Slyke procedure: oxygenating by gently rotating a thin layer of the blood sample, followed by gasometric determination of the oxygen. These results when compared with those obtained by the iron and acid hematin methods, upon samples removed at the third, fifth, and twelfth week disclosed no effect by the "chlorophyllin" upon the oxygen carrying capacity of the hemoglobin.

Gross Pathology.—Animals sacrificed at the fifth, fifty-second, and approximately the hundred and fourth week, exhibited the usual expected findings upon autopsy. These findings were distributed among the control as well as the test groups of animals. They comprised, in those animals sacrificed at two years, ventricular edema; consolidated areas in the lung; occasional liver tumor and occasional cystic areas; retention cysts and minor congestion of the kidneys; several pituitary tumors; hyperplasia of lymphoid tissue of the small intestine; small reproductive organs; all of these being findings consistent with the age and strain of animals.

Organ Weights.—At the time of sacrifice and autopsy of animals at the fifty-second and one hundred and fourth week, the weight of the principal organs was determined. These weights were calculated to the gram weight of the tissue per 100 Gm. weight of the test animal, and are reported herewith (Table V). There were no significant differences.

Plasma "Chlorophyllin" and Copper.—When potassium sodium copper chlorophyllin is fed to rats at a sufficient concentration in the diet, it is transmitted across the gastrointestinal membrane and appears in the plasma. Under such conditions the plasma is tinted and contains determinable amounts, when the diets of the animals contain more than 0.1% of the "chlorophyllin" for at least several days. The amount of "chlorophyllin" and of copper is variable and is governed by the concentration of the "chlorophyllin" in the food which is ingested (Table VI). For the purpose of determination, blood is collected into heparin by cannulation of an external carotid artery, and then gently centrifuged. The centrifuged plasma is separated, clarified by the addition of nine parts of ethyl alcohol which has been previously adjusted to a *p*H of 9 by the addition of sodium hydroxide. The centrifuged clear alcohol dilution of the plasma is then read at 400 mμ, against a similarly treated plasma from

TABLE V.—AVERAGE WEIGHT OF TISSUES, GRAMS PER 100 GM. OF BODY WEIGHT

	N	Heart	Lungs	Liver	Spleen	Kidneys	Uterus (Seminal Vesicles)	Ovaries (Testes)	Stomach	Brain	Approx. Weeks on Test
Females											
Controls	6	0.384	0.554	3.902	0.203	0.816	0.256	0.040	0.634	0.614	104
Potassium sodium copper chlorophyllin											
0.1% in diet	10	0.367	0.555	3.559	0.240	0.799	0.285	0.078	0.645	0.616	104
1.0% in diet	9	0.371	0.590	4.375	0.213	0.855	0.263	0.040	0.708	0.615	104
3.0% in diet	7	0.389	0.670	3.632	0.232	0.953	0.346	0.042	0.758	0.712	104
Males											
Controls	4	0.358	0.526	3.564	0.208	0.857	0.227	0.737	0.661	0.488	104
Potassium sodium copper chlorophyllin											
0.1% in diet	6	0.415	0.532	3.946	0.179	0.872	0.189	0.688	0.686	0.584	104
1.0% in diet	5	0.366	0.704	4.419	0.177	1.190	0.193	0.537	0.725	0.506	104
3.0% in diet	4	0.347	0.404	4.015	0.176	0.921	0.225	0.699	0.770	0.506	104
Females											
Controls	9	0.317	0.500	3.214	0.203	0.717	0.274	0.038	0.615	0.656	42
Copper sulfate (530 p. p. m. Cu)	15	0.295	0.553	3.250	0.182	0.714	0.212	0.037	0.628	0.630	42
(1,600 p. p. m. Cu)	10	0.301	0.564	3.778	0.209	0.799	0.179	0.040	0.821	0.684	42
Copper gluconate (1,600 p. p. m. Cu)	4	0.329	0.554	4.825	0.255	0.894	0.078	0.024	1.127	0.821	42
Males											
Controls	8	0.268	0.495	3.586	0.169	0.798	0.827	0.350	0.518	0.424	42
Copper sulfate (530 p. p. m. Cu)	12	0.282	0.487	3.674	0.189	0.792	0.666	0.357	0.585	0.423	42
(1,600 p. p. m. Cu)	6	0.301	0.488	4.072	0.198	0.889	0.839	0.405	0.686	0.505	42
Copper gluconate (1,600 p. p. m. Cu)	2	0.419	0.967	3.940	0.198	1.052	0.760	0.157	1.227	1.136[a]	42
Females											
Controls	4	0.336	0.770	3.524	0.188	0.753	0.230	0.039	0.645	0.668	33
Copper sulfate (1,600 p. p. m. Cu)	4	0.333	0.569	3.767	0.185	0.670	0.135[b]	0.024[b]	0.795	0.669	33
Copper gluconate (1,600 p. p. m. Cu)	4	0.378	0.676	4.465	0.215	0.782	0.081	0.022	1.029	0.648	33
Males											
Controls	4	0.301	0.713	3.556	0.173	0.777	0.923	0.359	0.531	0.479	33
Copper sulfate (1,600 p. p. m. Cu)	4	0.297	0.518	3.492	0.176	0.720	0.700	0.255	1.061	0.572	33
Copper gluconate (1,600 p. p. m. Cu)	4	0.328	0.553	3.963	0.205	0.891	1.008	0.286	1.013	0.664	33

control animals, in a Beckman model DU Spectrophotometer, using a slit width of 0.08 mm.

By trial it was found possible to recover 60% of "chlorophyllin" added to plasma obtained from control rats after this plasma with the added "chlorophyllin" was clarified in the manner described. A concentration of 25 μg./ml. was detectable and the recovery uniform over a range of 30 μg. to 150 μg./ml. (corrected). The separated precipitated plasma fractions are of a greenish hue, and the chlorophyllin is firmly bound therein. An assumption of course, enters into the conclusion, namely, that the chlorophyllin read in the plasma fraction has the same spectral characteristics as that added to plasma obtained from control animals.

Other methods of clarifying the plasma to make the readings possible were not as successful, inasmuch as the "chlorophyllin" in the plasma is readily precipitated at lower *p*H values.

The copper content of the plasma is not in agreement with that which should be present by reason of the "chlorophyllin" content. A copper content of 615 μg./100 ml. over and above that present normally in the plasma should be found when "chlorophyllin" is present to the extent of 116 μg./ml. The excess copper found was approximately 100 μg./100 ml. Upon the lower diet intake of "chlorophyllin" excess copper was not observed. Copper was determined by the method of Gubler *et al.*, (23).

Fecal "Chlorophyllin."—Though "chlorophyllin" as ingested is soluble in water, a 1% solution having a *p*H of 9.7, is precipitated upon the addition of acid, and redissolved in the presence of alkali. However, "chlorophyllin" excreted in the feces is no longer soluble either in water or in mild alkali. In fact the feces, though vividly green, are not completely extractable by organic solvents and it is only upon treatment with glacial acetic acid and repeated extraction with ether that an appreciable quantity of the "chlorophyllin" is removed in the separated ether fractions. The remaining feces are still distinctly green in color. These ether extractions were subsequently partitioned between 5, 10, 25, and 37% HCl according to the usual methods (24). The acid extractions are subsequently neutralized with sodium hydroxide-sodium acetate, re-extracted with ethyl ether, and the ether solution of the acid fractions then read spectrophotometrically in the ultraviolet and visible range on a Beckman model DU. A sample of 100 mg. of dried feces was extracted in this manner. A quantity of "chlorophyllin" was subjected to the same procedure and read in the same manner for comparison. The curves of transmittance in the ultraviolet range of the several "chlorophyllin" acid fractions obtained from feces did not differ materially from the curves resulting when "chlorophyllin" itself is treated in the same way (Fig. 4). The curves obtained in the visible range for the fecal "chlorophyllin" acid fractions as regards that portion of the "chlorophyllin" which is *not* extractable by acid show a shift in the maximum peaks of absorption from 520–540 mμ and 620–660 mμ (Fig. 5). The rest of the fecal acid fractions are comparable in the visible range with the direct "chlorophyllin" fractions.

The 5% acid fractions which would include both porphyrins and phylloerythrin, are fluorescent; the 10% acid fractions which might include phylloerythrin are nonfluorescent. However, there is no phylloerythrin absorption peak at 520 mμ, in either the 5% or 10% HCl fractions both of which extract phylloerythrin.

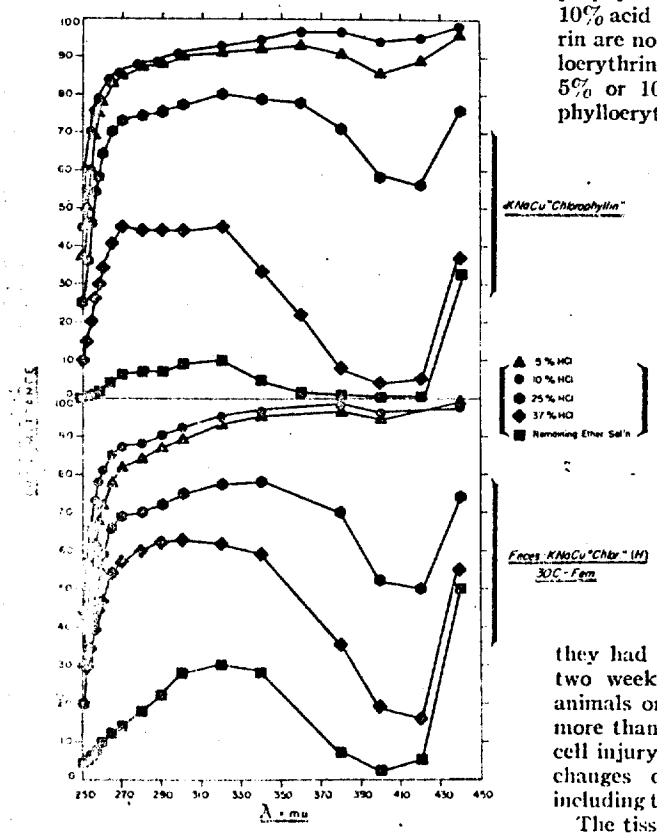

Fig. 4

Ingested "chlorophyllin" is excreted in the feces essentially in an insoluble form, probably occurring as a calcium complex which is difficult to cleave. Copper is present, apparently tightly bound to the chlorophyll moiety.

Tissue-Stored Copper and Iron.—An examination was made of the liver, kidneys, and spleen of animals sacrificed at the tenth, fifty-second, and one hundred and fourth week, for the copper and iron content, of the tissues. These findings will be discussed in the section *Tissue-Stored Copper and Iron* under that part referring to the administration of other copper compounds.

Histopathology. — The kidneys, liver, stomach, small intestine, and spleen of animals sacrificed after they had been in the study for a period of fifty-two weeks were examined. The livers of those animals on the high level (3%) intake showed no more than tinctorial changes without indication of cell injury. All other tissues disclosed only minor changes distributed among the several groups including the controls.

The tissues of those animals in the control group and the high level (3%), which were sacrificed at the

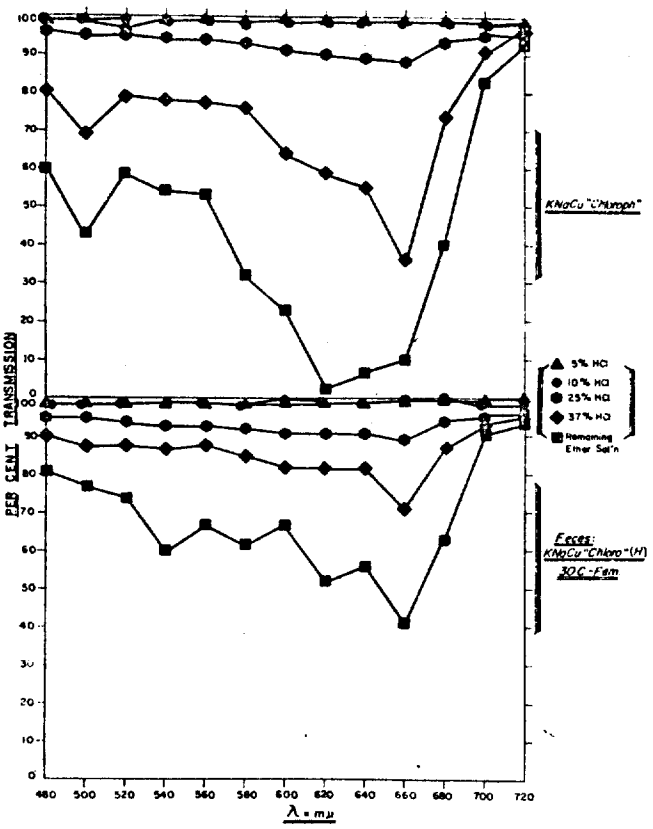

Fig. 5

TABLE VI.—"CHLOROPHYLLIN" AND COPPER CONTENT OF PLASMA FROM ADULT MALE RATS AFTER 62 DAYS ON THE SPECIFIED DIET

Data from Pooled Samples of Plasma from 3 Rats on Each Level

Diet	"Chlorophyllin," μg./ml.	Copper, μg./100 ml.
Control diet	None	189
"Chlorophyllin"		
0.1%	None	174
1.0%	58	196
3.0%	116	303

termination of the study (one hundred and four weeks) were examined with findings as follows: *Kidneys.*—All sections of the control and test animals showed interstitial scarring, tubular atrophy, dilated tubules filled with hyaline material and minor inflammatory changes. It appears that the changes are related to the age of the animals rather than to the product. *Livers.*—All livers were well within the normal limits. *Spleen.*—All spleens were within normal limits. *Adrenals.*—Changes of a cystic and old hemorrhagic nature in the cortex of two high level (3%) animals and a small adenoma in an animal of the same level were observed. *Testicles and Seminal Vesicles.*—These were normal in all animals. *Ovaries and Uterus.*—These showed a normal histology when compared with their controls. *Gastrointestinal Tract.*—Those sections taken from the upper stomach, including a portion of the esophagus, from the duodenum with a fragment of pancreas attached, and from the large intestines showed in both the control and test animals a normal histopathology especially with respect to the mucosa and submucosa where pathology if present would show itself most prominently. *Nerve Tissue (Sciatic).*—There is no evidence that the material given, either changes the nerve sheath histologically or gives rise to fibrotic or a chronic inflammatory state. *Heart.*—The test animals compared with the controls showed no particular change.

SUMMARY

Aside from minor adrenal cortical changes there was no evidence of adverse effects of the administered substance upon the organs examined. The cortical changes in themselves could well be associated with old age.

LIFE CYCLE TOXICITY STUDY (CHRONIC STUDY) ON RATS OF OTHER COPPER COMPOUNDS

Though potassium sodium copper "chlorophyllin" as marketed contains between 4 and 5% of copper, this copper appears to be rather firmly bound. About 0.25% of copper can be recovered as soluble copper when "chlorophyllin" is dissolved in water and the solution adjusted to a *p*H of 3, by the slow addition of hydrochloric acid, then filtered. Thus better than 90% of the copper appears to be firmly bound in the molecule from which it is not released by hydrochloric acid at a *p*H of 3. Under the same experimental conditions, both copper sulfate and copper gluconate yield all of their copper.

When 3% of the "chlorophyllin" is incorporated in the diet, correcting by the presently conventional used assay procedure for "chlorophyllin," this diet contains 1,600 p. p. m. of copper, a concentration of copper which, if administered as copper sulfate, is known to retard growth of the rat and result in metal toxicity. However, growth retardation was not observed among animals which received "chlorophyllin" in a preliminary study; this, together with the evidence that copper is not at least released appreciably *in vitro*, supported an assumption that the majority of the copper in potassium sodium copper chlorophyllin might not be available to the animal. However, to confirm this, a secondary study was initiated wherein weanling Sprague-Dawley rats were placed upon diets containing copper sulfate equivalent in copper content to the copper content of the 3% and 1% potassium sodium copper chlorophyllin diets. These levels corresponded to 1,600 p. p. m. and 530 p. p. m. respectively of copper as copper sulfate in the diets. A similar group of animals was placed upon a diet containing copper in the form of gluconate equivalent to 1,600 p.p.m. copper in the diet. These concentrations were moving concentrations, i. e.

TABLE VII.—AVERAGE BODY WEIGHTS OF RATS RECEIVING COPPER SULFATE OR COPPER GLUCONATE IN THE DIET

		0 week	12th week	8th week	12th week	20th week	35th week
				Females			
Controls	N	75 ± 2.3 Gm.[a] 25	172 ± 3.2 Gm. 24	204 ± 4.0 Gm. 24	220 ± 3.9 Gm. 24	291 ± 4.5 Gm. 24	265 ± 4.3 Gm. 24
Copper sulfate (anhyd.) 0.135% in diet	N	67 ± 3.3 25	154 ± 2.8 25	207 ± 3.5 25	232 ± 3.2 25	270 ± 3.5 25	200 ± 5.1 25
0.400% in diet	N	73 ± 2.2 25	153 ± 3.4 25	198 ± 2.7 25	224 + 3.1 25	220 ± 4.2 24	257 ± 3.6 20
Copper gluconate 1.147% in diet	N	75 ± 2.5 25	170 ± 2.9 25	200 ± 3.1 25	235 ± 4.1 25	294 ± 3.8 23	182 ± 11.7 6[b]
				Males			
Controls	N	81 ± 2.3 23	218 ± 7.2 23	310 ± 6.2 23	382 ± 7.0 23	438 ± 17.3 23	459 ± 17.3 22
Copper sulfate (anhyd.) 0.135% in diet	N	72 ± 3.4 25	194 ± 6.5 25	279 ± 4.3 25	358 ± 5.8 25	425 ± 10.7 24	431 ± 3.7 23
0.400% in diet	N	71 ± 9.3 23	174 ± 5.7 23	247 ± 6.3 23	280 ± 9.1 23	282 ± 10.6 20	335 ± 9.5 10
Copper gluconate 1.147% in diet	N	75 ± 2.7 22	198 ± 7.5 22	272 ± 7.6 22	327 ± 6.5 22	268 ± 8.3 15[b]	210 ± 11.0 2

[a] Standard Error.
[b] Depleted due to high mortality.

25%, 50% and then 100% of the stated amount after twenty-eight days, as described under the "chlorophyllin" study. The several levels of the "chlorophyllin" and the other copper salts are keyed in the figures as: high (H)—1,600 p.p.m. Cu, middle (M)—530 p.p.m. Cu, and low (L)—53 p. p. m. Cu. A control group was maintained concurrently on the basic diet which, as in the previous study, was Rockland rat meal. Each group contained fifty animals, equally divided between the sexes.

Growth and other data accumulated from this study may be compared with similar data gathered in the life cycle study on potassium sodium copper chlorophyllin.

Growth (Weight Gain).—Animals on the high level of copper sulfate and those upon copper gluconate were adversely affected in growth (Table VII). This retardation became readily discernible at the twenty-sixth week, when the male control animals and the animals receiving 530 p. p. m. of copper as copper sulfate weighed at least 50% more than those animals upon the 1,600 p. p. m. copper intake, either as sulfate or as gluconate (Fig. 6). Animals upon 1,600 p. p. m. copper as potassium sodium copper chlorophyllin were not affected in regard to weight gain. The adverse effect of copper as gluconate upon growth was the most marked. However, the lower growth rate is not due entirely to a toxic effect, at least during the first twelve weeks, as the total intake of food was less and the gain in weight per gram of food consumed was similar for all groups (Table IV). The accentuated toxic effect of copper in this form was also discernible in the death rate of this group between the fourth and eighth month, during which time nearly 90% of the animals died (Fig. 3). Because it was feared all of the copper gluconate animals would be lost, four animals of the control group, the high level copper sulfate, and the copper gluconate groups were sacrificed shortly thereafter. The balance of the animals were continued in the study and all surviving animals of all groups were sacrificed at the fortieth to forty-fourth week.

Blood and Urine Examinations.—Routine hematologic and urine examinations were performed at intervals. All factors were within normal expected ranges, except blood nonprotein nitrogen levels. High NPN was noted in the males.

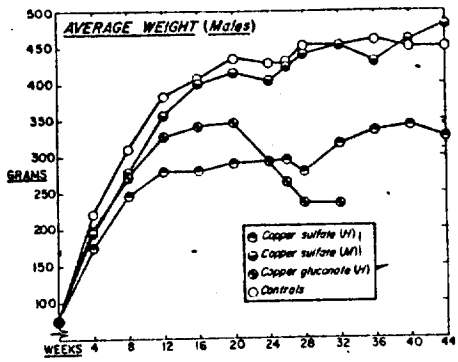

Fig. 6

being 83 mg. % for the animals receiving copper sulfate (1,600 p. p. m. Cu), and 109 mg. % for the copper gluconate (1,600 p. p. m. Cu) animals. The lower level copper sulfate male animals, and the female animals on all levels of copper were just above the expected range of 50-70 mg. % of non-protein nitrogen.

Oxygen Carrying Capacity.—Gasometric determinations of the oxygen carrying capacity of the blood compared satisfactorily with the hemoglobin value determined by the iron and acid hematin methods.

Organ Weights.—The average weight of the various organs per 100 Gm. weight of animal, were within the expected ranges as compared to the controls of the same age, except certain tissues. Animals receiving copper gluconate had hypertrophied uteri, ovaries, or seminal vesicles. The stomachs of female animals on the high level of copper sulfate and those of the females and males receiving the diet containing copper gluconate were enlarged (Table V).

Gross Pathology.—A number of animals were sacrificed between the thirtieth and thirty-fifth week to avoid the possible loss of all animals receiving copper gluconate. All surviving animals were sacrificed during the fortieth to forty-fourth week. Aside from findings that were distributed throughout all the groups and expected in the animals of the indicated age, the following were common to the test groups upon the higher intake of copper salts: Bronzed kidneys exhibiting sharp demarcation between the cortex and the medulla; bronzed or yellowish livers; hypertrophied ridges between the cardiac and peptic portions of the stomach, occasional ulcer, some blood; bloody mucous in the intestinal tract. The stomachs of some of these animals receiving copper gluconate were often flabby and distended. These observations were noted especially among the animals upon the high intake of copper sulfate and copper gluconate, which is in

TABLE VII.—COPPER CONTENT OF TISSUES OF RATS RECEIVING POTASSIUM SODIUM COPPER CHLOROPHYLLIN IN DIET, MG. Cu/100 GM. TISSUE (WET BASIS)

	Control Diet		Potassium Sodium Copper Chlorophyllin					
			0.1%		1.0%		3.0%	
	M	F	M	F	M	F	M	F
Liver								
10 weeks								
Av.	0.41	0.48	0.47	0.57	0.58	0.74	0.56	0.56
S.E.	0.04	0.08	0.026	0.09	0.035	0.065	0.06	0.08
N.	4	4	4	4	4	4	4	4
52 weeks								
Av.	0.78	1.09	1.46	1.14	0.81	2.43	1.06	2.14
S.E.	0.020	0.052	0.64	0.29	0.064	1.12	0.42	0.711
N.	3	3	3	3	3	3	3	3
104 weeks								
Av.	1.82	1.10	1.47	1.85	1.85	2.02	2.18	3.71
S.E.	0.58	0.152	0.304	0.251	0.504	0.51	0.61	1.28
N.	4	6	6	10	5	9	4	7
Kidney								
10 weeks								
Av.	1.07	1.72	1.47	1.52	1.58	1.57	1.48	1.65
S.E.	0.15	0.57	0.27	0.11	0.51	0.16	0.32	0.22
N.	4	4	4	4	4	4	4	4
52 weeks								
Av.	2.08	4.46	1.52	2.44	1.83	3.79	2.11	2.97
S.E.	0.17	2.20	0.27	0.55	0.364	0.847	0.015	0.11
N.	3	2	3	3	3	3	3	3
104 weeks								
Av.	3.45	2.25	2.03	2.55	2.35	3.19	2.48	3.22
S.E.	0.91	0.23	0.709	0.19	0.727	0.393	0.63	0.416
N.	4	6	5	10	5	9	4	6
Spleen								
10 weeks								
Av.	0.96	1.59	0.52	0.46	0.40	0.72	0.68	0.52
S.E.	0.42	0.05	0.30	0.03	0.48	0.38	0.11	0.18
N.	2	2	2	2	2	2	2	2
52 weeks								
Av.	1.83	4.00	2.92	3.26	3.05	3.46	2.36	3.61
S.E.	0.58	1.02	1.45	1.02	1.36	0.817	1.03	1.80
N.	2	3	3	3	3	3	2	3
104 weeks								
Av.	3.38	6.96	3.34	1.92	2.75	2.34	3.01	2.96
S.E.	1.44	2.22	0.408	0.396	0.513	0.386	0.775	0.[illegible]
N.	4	6	6	10	5	9	4	7

TABLE IX.—IRON CONTENT OF TISSUES OF RATS RECEIVING POTASSIUM SODIUM COPPER CHLOROPHYLLIN IN DIET, MG. Fe/100 GM. TISSUE (WET BASIS)

	Control Diet		Potassium Sodium Copper Chlorophyllin					
			0.1%		1.0%		3.0%	
	M	F	M	F	M	F	M	F
Liver								
10 weeks								
Av.	2.36	2.50	2.25	3.56	1.57	2.26	3.22	2.09
S.E.	0.83	0.27	0.12	0.43	0.53	0.37	0.59	0.82
N.	2	2	2	2	2	2	2	2
52 weeks								
Av.	2.64	7.79	2.14	11.20	5.15	7.83	3.17	8.76
S.E.	0.458	0.341	0.258	1.92	2.50	2.35	0.441	0.452
N.	3	3	3	3	3	3	3	3
104 weeks								
Av.	17.7	24.7	16.6	27.0	15.7	24.9	18.0	31.3
S.E.	1.9	8.44	2.32	3.67	2.94	3.90	2.85	6.45
N.	4	6	6	10	5	9	4	7
Kidney								
52 weeks								
Av.	7.45	11.22	10.82	16.76	13.86	19.69	10.73	16.21
S.E.	1.32	1.95	2.40	3.59	2.40	1.01	2.41	2.67
N.	3	2	3	3	3	3	3	3
104 weeks								
Av.	19.9	32.4	24.7	25.6	17.4	31.1	23.5	28.3
S.E.	1.4	7.7	1.88	2.12	4.0	2.83	2.61	4.88
N.	4	6	6	10	5	9	4	6
Spleen								
104 weeks								
Av.	219.0	229.4	162.6	190.9	160.5	206.8	235.4	279.6
S.E.	24.6	32.7	30.4	17.3	27.5	27.9	13.0	41.2
N.	4	6	6	10	5	9	4	7

contrast to the relatively normal findings observed among animals receiving potassium sodium copper "chlorophyllin" contributing the same concentration of copper.

Histopathology.—Histopathological studies were performed on the organs of test animals receiving the high level of copper sulfate and gluconate, which were sacrificed after thirty to thirty-five weeks and also on the liver, kidney, and testes of animals receiving the lower level of copper sulfate sacrificed after forty to forty-four weeks. The results of these studies indicated the following organs to be normal in all the test animals as well as the controls: spleen, adrenals, small intestines, large intestines, stomach, and sciatic nerve. Kidney sections of those on the high level of copper sulfate and gluconate, did, however, show minor changes which did not correlate well enough throughout the animals to draw any definite conclusion. A study of liver sections of the animals receiving these copper salts revealed well defined abnormalities of a toxic nature in both the males and the females in that their icteric pigmentation was increased and cytoplasmic staining properties were abnormal.

Varying degrees of testicular degeneration were noted in both the high and low levels of the copper sulfate animals; the ovaries of the females on this product were not noticeably affected to any degree. The kidneys, liver, and testes of all the control animals were found to be normal.

Tissue-Stored Copper and Iron.—The liver, kidneys, and some spleens of animals from all groups were examined as to their total copper and iron content (Tables VIII to X).

Liver copper averaged less than 2 mg. per 100 Gm. of tissue in those animals receiving the control diet, and the two diets containing 0.1% and 1.0% of potassium sodium copper chlorophyllin. Those animals receiving 3% of "chlorophyllin" had a somewhat higher liver copper content which was non-significant and which concentration was noted only after the animals had been on the diet for a period of two years. Animals receiving 530 p. p. m. of copper in the form of sulfate, stored in forty weeks, 12 mg. to 32 mg. per 100 Gm. of liver, i. e., a storage of more copper than the high level "chlorophyllin" animals exhibited in a period of two years. Furthermore, the liver of animals which received 1,600 p. p. m. of copper as copper sulfate had deposited in them 38-46 mg. of copper per 100 Gm of tissue (Fig. 7). Comparing these figures with the copper

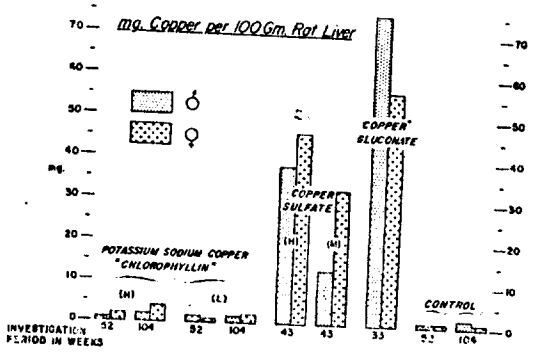

Fig. 7

found in the liver of animals receiving 1,600 p. p. m. of copper as gluconate, there is even a more marked difference, i. e., the liver of these latter animals

TABLE X.—COPPER AND IRON CONTENT OF TISSUES OF RATS RECEIVING COPPER SULFATE OR COPPER GLUCONATE IN DIET, MG. Fe OR Cu/100 GM. TISSUE (WET BASIS)

	Control Diet		Copper Sulfate, Cu 530 p. p. m.		Copper Sulfate, Cu 1,600 p. p. m.		Copper Gluconate, Cu 1,600 p. p. m.	
	M	F	M	F	M	F	M	F
Copper Content								
Liver								
Av.	1.16	1.78	12.47	32.36	38.28	45.77	75.1	56.6
S.E.	0.31	0.39	2.52	14.6	13.85	5.18	12.07	6.1
N.	6	6	6	5	6	6	6	6
Kidney								
Av.	2.48	3.53	3.49	6.91	15.83	12.11	59.57	51.1
S.E.	0.20	0.33	0.54	0.48	6.21	4.80	14.75	21.5
N.	6	6	6	6	6	6	5	5
Spleen								
Av.	3.34	4.83	5.63	5.12	13.91	6.07	12.39	13.77
S.E.	0.63	0.33	1.5	1.3	7.50	1.72	3.9	3.29
N.	6	6	6	6	6	6	6	6
Iron Content								
Liver								
Av.	9.7	14.74	18.0	16.5	14.1	10.5	5.9	8.5
S.E.	2.5	4.0	9.6	1.6	6.3	5.2	2.3	5.0
N.	6	6	6	5	6	6	6	6
Kidney								
Av.	16.4	17.44	12.6	15.0	11.8	14.8	10.6	9.0
S.E.	1.4	1.74	1.97	0.98	1.7	1.5	1.2	2.0
N.	6	6	6	6	6	6	5	5
Spleen								
Av.	128.1	191.7	120.3	292.1	108.9	148.7	49.7	86.1
S.E.	18.9	37.3	13.6	12.4	18.7	41.7	11.4	41.7
N.	6	6	6	6	6	6	6	6

stored 56–75 mg. of copper per 100 Gm. of tissue, a concentration nearly twice that produced by administering an equivalent amount of copper sulfate and attained in less time, i. e., in approximately forty weeks *vs.* thirty weeks.

Liver storage of copper appears not to be significantly different between the male and female inasmuch as the high averages, as they occur, invert between the sexes.

The high storage of copper by the copper gluconate animals correlates with the high death rate of these animals and the high blood nonprotein nitrogen. There is also marked evidence among these animals of unfavorable gross pathology and histopathology.

In the kidneys (Fig. 8) and the spleen (Fig. 9)

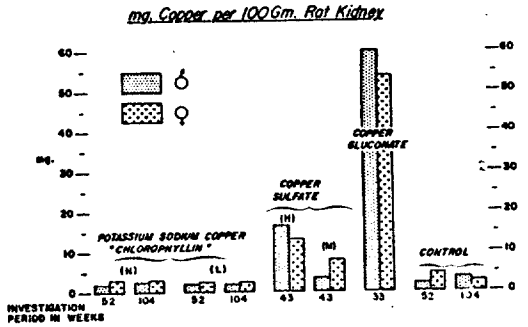

Fig. 8

animals receiving in the diet as high as 3% "chlorophyllin" (1,600 p. p. m. Cu) stored no more copper than animals on the control diet. There is, however, a somewhat higher storage of copper in the tissues

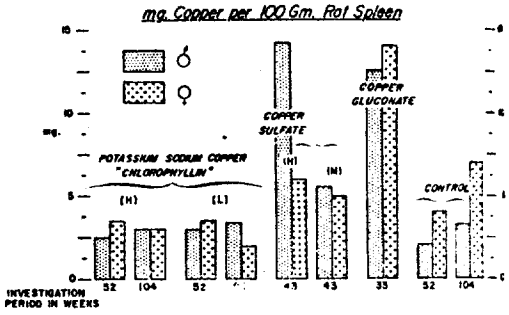

Fig. 9

of those animals receiving copper sulfate (530 p. p. m. Cu), which is accentuated among the animals receiving the high level of copper sulfate (1,600 p. p. m. Cu); furthermore, in the kidneys of those animals receiving copper gluconate (1,600 p. p. m. Cu), there was deposited nearly twenty times the quantity of copper found in the tissues of the control animals.

Concurrently with the examination of the tissues for their copper content, a determination of the iron content was made. Iron storage does not differ between the controls and those animals receiving "chlorophyllin" in the diet, though iron storage in the liver appears to be somewhat greater in the female (Fig. 10). However, when there is a high storage of copper as in the instance of the animals receiving copper sulfate and copper gluconate, this appears to prevent in part or depress the storage of iron (Table X).

A similar situation exists in the kidneys, and the females again appear to store generally a greater

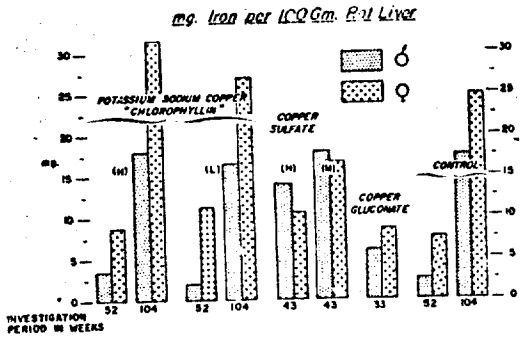

Fig. 10

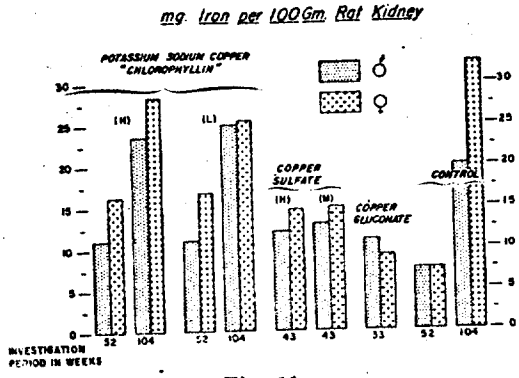

Fig. 11

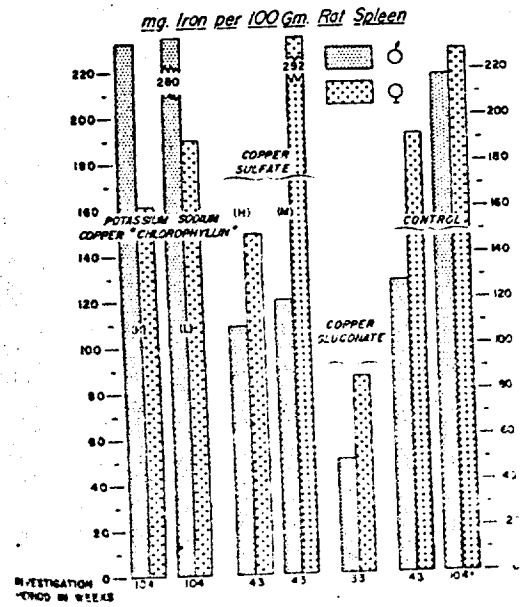

Fig. 12

quantity of iron (Fig. 11). Storage of iron in the female spleen is greater than in the male and is apparently markedly depressed by the storage of excess copper (Fig. 12).

An increased iron storage in all tissues is strikingly evident in animals of greater age.

Tissues were dry ashed at 525°, the copper determined by the diethylthiocarbamate procedure, and the iron by a modified *o*-phenanthroline procedure (Methods, A. O. A. C., Seventh Edition).

Effect Upon Availability of Ascorbic Acid.—Traces of copper accentuate the oxidation of ascorbic acid, and copper when bound as a protein complex is still effective in accentuating this oxidation. This suggested that a high level of copper in the diet as potassium sodium copper chlorophyllin or of other copper salts might result in a vitamin C deficiency. An attempt was made to feed dry mix diets containing "chlorophyllin," copper sulfate, or copper gluconate, to guinea pigs, but the animals refused diets containing copper sulfate. By trial it was found that guinea pigs would not refuse these materials when supplied in water up to a copper content equivalent of 0.5% "chlorophyllin," (260 p. p. m. Cu). Subsequently 20 young female pigs weighing 150 to 200 Gm. each, were divided into four groups: one group was maintained as a control, the other groups received copper sulfate, copper gluconate, or "chlorophyllin" in all of their fluid intake (water). The animals were maintained for eleven weeks on a diet reasonably adequate in vitamin C, comprised of Rockland guinea-pig pellets and 70-75 Gm. of fresh cabbage per week. Food and water consumption did not markedly differ except in the first few weeks, during which the animals on the copper sulfate solution did not drink as freely. There was no clinical evidence of scurvy in any of the animals; no significant difference in survival; weight gain was better in the "chlorophyllin" animals, and not greatly different in the animals of the other groups. However, animals receiving copper sulfate and copper gluconate did not gain weight as rapidly during the initial period of test. At the termination of the eleventh week all animals were sacrificed and found to be without gross evidence of scurvy. Blood ascorbic acid was determined before sacrificing; there was no significant difference between the average figures of the several groups (Table XI).

TABLE XI.—ASCORBIC ACID CONTENT OF SERUM OR WHOLE BLOOD, mg. %

Diet	Rat Blood Serum Males	Rat Blood Serum Females	Guinea-Pig Blood, Females
"Chlorophyllin, 3%" (1,600 p. p. m. Cu)	0.43	0.40	0.96
Copper sulfate (1,600 p. p. m. Cu)	0.48	0.39	0.95
Copper gluconate (1,600 p. p. m. Cu)	0.12 0.55	0.57	1.05
Control (2 p. p. m. Cu)	0.47	0.39	0.90

Average of 5 rats, except copper gluconate where individual figures for 2 male rats is given. Average of 4 animals for guinea pigs.

Though rats do not require ascorbic acid in their diet, animals after being on the "chlorophylllin" diets for one year, and the copper sulfate and gluconate diets for forty weeks, were examined for serum ascorbic acid. No difference from the normal range

for rats was found, except that one of two animals which were receiving copper gluconate had a low serum ascorbic acid level.

PHOTOSENSITIZATION

To establish whether potassium sodium copper chlorophyllin would cause photosensitization if it were present as such in the blood or was degraded into sensitizing porphyrins, several routes of administration were followed and several means of excitement; that is, prolonged high intensity light (photoflood), short high intensity light (photoflash), and prolonged ultraviolet exposure (quartz mercury arc). Guinea pigs, mice, and rats were subjected to exposure by these means after having received the "chlorophyllin" by direct application to the skin, oral intake, intraperitoneal, or intravenous injection. Only albino animals were employed and these were in some instances shaved and washed before exposure. Neither general shock, pustulation, edema, or erythema developed except that erythema was produced by the quartz mercury arc in shaved animals upon prolonged exposure, including the controls. The guinea pig, which is quite susceptible to sensitization by many substances, was not abnormal in response even after repeated application of the material. Furthermore, animals which had received as much as 3% of "chlorophyllin" in their diet for over a year, responded no differently than did control animals. These experimental procedures failed to produce photosensitization.

DISCUSSION

Many investigators have shown that the administration of copper in any considerable dosage to the albino rat is followed by metal toxicity, an increased storage of copper, more especially in the liver, kidney and spleen, damage to these organs, and a high mortality. We have confirmed these findings and observed that copper, when organically combined as the gluconate, either more readily passes across the gastrointestinal membrane or is protected against inactivation by proteins, lipids, etc., present in the food. Thus it is stored more rapidly and to a greater extent, resulting in an increased mortality. Copper or other metallic salts may in this form offer a therapeutic advantage.

Equivalent daily amounts of copper administered as the complex potassium sodium copper chlorophyllin do not cause metal toxicity even when given over a more prolonged period. There is no storage of copper in the liver, kidney, or spleens of such animals comparable to that resulting subsequent to the administration of copper in the form of sulfate or gluconate. Therefore, though excessive amounts of copper cause liver damage when given in the form of a salt such as the sulfate or gluconate, or as powdered metallic copper, the same amount of copper complexed with "chlorophyll" is innocuous. This copper is not available for deposit in the tissues, though the chlorophyllin complex is transported in the plasma, when a sufficient concentration of the material is fed in the diet. However, the full complement of copper for the amount of "chlorophyllin" in the plasma is not found. Only about 1/6 of the theoretical copper is present.

When there is a high intake of available copper as sulfate, etc., as distinguished from the firmly bound copper of "chlorophyllin" there is an over all high level of storage in the tissues. However, there also appears a great many individual peculiarities. An occasional animal may store a tremendous quantity in some one tissue, such as the liver, yet another animal on the same intake may store no more copper than a control animal on a blank diet. For instance, copper storage in the livers of the group on copper sulfate (530 p. p. m. Cu) range from 1-79 mg. copper per 100 Gm. of tissue. Neither an excessively high storage, nor an inordinately low storage of copper follows in other tissues of the same animals. There is therefore some individual peculiarity of a particular tissue that plays an unexplained role.

Corwin (8) in commenting upon photosensitization suggested that effective amounts of chlorophyll may not enter the blood stream and Zirm and Kilebes (9) found no evidence of water-soluble chlorophyll derivatives in the blood or tissues when C^{14} labeled sodium magnesium chlorophyllin was administered orally. We have shown by spectrophotometric examination that at least a portion of the chlorophyll complex does enter the blood when a sufficient concentration is fed in the diet. However, photosensitization, which is often attributed to porphyrins did not follow and it was not possible to develop light sensitive animals by other means of administration of the material.

Blum (25) also observed that the presence of porphyrins does not necessarily indicate sensitivity to light and that sensitivity to light is not always accompanied by the presence of porphyrins.

Sensitizing porphyrins are absent though porphyrins are extracted from the 5 and 10% HCl fecal fractions as evidenced by fluorescence in the ultraviolet region following the procedure of Brugsch and Sheard (26). But phylloerythrin appears to be absent, as absorption peaks do not occur at 520 mμ (Fig. 5). Furthermore, the porphyrin content of the feces from rats upon the control diet is not materially higher than that of the rats receiving 3% of the "chlorophyllin."

Potassium sodium copper chlorophyllin which is water-soluble in its original form is excreted in the feces in a water-insoluble and essentially organic solvent insoluble form. It has apparently exchanged its potassium and sodium and substituted calcium. Alkali does not reconvert it and the copper apparently remains complexed within the structure. This may in part explain the excellent survival over a period of nearly two years, of animals receiving "chlorophyllin" not withstanding the inordinately high amount of the copper complex which was purposely fed. Survival was even better than the survival of those animals on the blank or control diet, and may be compared with the 90% mortality of male rats upon copper gluconate, and high mortality among the high level copper sulfate animals, all of which substantiate the essential non-toxicity of the potassium sodium copper chlorophyllin.

We presume it is by reason of the firmness of the copper complex that a high intake of copper in this form is innocuous. Further, the complexed copper not being readily removed from within the structure, the conversion of the potassium sodium com-

chlorophyllin as ingested to other chlorophyll fractions, is blocked. As the magnesium in chlorophyll or in a true chlorophyllin is readily removed, this physical-chemical blocking may not exist for these compounds.

Potassium sodium copper chlorophyllin is, under these circumstances, apparently essentially excreted as an insoluble calcium copper chlorophyllin.

SUMMARY

The inclusion of up to 3 per cent of potassium sodium copper chlorophyllin in the diet of albino rats over their life span resulted in no indication of toxicity, in that:

1. The growth rate, survival, blood and urine factors, and ability to conceive, were normal.

2. There was no gross or microscopic pathology attributable to the "chlorophyllin."

3. There was no evidence of metal toxicity, and the livers, kidneys, and spleens, did not store increased amounts of copper, compared with that stored by similar tissues of animals receiving equilivalent quantities of copper sulfate or copper gluconate.

4. Photosensitization did not occur, nor was it possible to incite sensitization by intraperitoneal or local application of the "chlorophyllin."

During the study it was also observed that:

5. Plasma contains "chlorophyllin" when a sufficient amount is fed in the diet.

6. Plasma contains additional copper under the same circumstances, but only to the extent of about 1/6 of that theoretically expected.

7. Copper gluconate is more readily absorbed and deposited in the tissues when administered orally than is an equivalent amount of copper as the sulfate.

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WHO MAKES IT?

The Registry of Rare Chemicals, Armour Research Foundation of the Illinois Institute of Technology, 35 West 33rd Street, Chicago 16, Ill., seeks information on sources of supply of the following chemicals:

Aluminum nitride
Silver hyponitrite
Sodium telluride
Phosphonium iodide
Diethylgermanium iodide
1,4-Cyclohexanediamine
Anthracene-9,10-dicarboxylic acid
2-Chlorocyclopentene-1
7-Nitrohydrindene
2,3,5,6-Tetramethylbenzoic acid
2,5-Dihydroxyphenylalanine
2,4,2′,4′-Tetrahydroxydiphenyl
2,3-Dichlorobenzyl alcohol
Epifluorohydrin
10-Methyl-1,2-benzanthracene
β-Chlorolactic acid
Murexine
Tangeretin
Nobiletin
Muscarine

Kirchgessner M-etal zeitschrift Fur Tierphysiologie 13 1967

Copper Absorption With Addition of Gluconic, Citric, Salicylic, and Oxalic Acid.

7th Report

The Synamics of Copper Absorption.

by M. Kirchgessner, U. Weser and H. L. Müller

The absorption of complexed copper were investigated in previous reports. It had been shown that the rate of copper absorption depended upon the complex size, the stability of the complex, as well as upon the specific absorption behavior of the complex ligand. In the present study, it was to be determined in how far the absorption of copper is influenced by the addition of free acids to the diet. For this purpose, several nitrogen free organic acids were chosen which are also present in higher concentrations in certain fodder or food substances.

Experimental Part.

50 white female Sprague-Dawley rats with an average body weight of 37 g were depleted for 17 days with a copper deficient diet in glass-plastic cages. Subsequently, the animals were divided into the following ten groups:

No Cu addition
Cu sulfate

$CuSO_4$ + 25 mg gluconic acid	$CuSO_4$ + 250 mg gluconic acid
$CuSO_4$ + 25 mg citric acid	$CuSO_4$ + 250 mg citric acid
$CuSO_4$ + 25 mg salicylic acid	$CuSO_4$ + 250 mg salicylic acid
$CuSO_4$ + 25 mg oxalic acid	$CuSO_4$ + 250 mg oxalic acid

The copper concentration was always 50 µmol per kg of fodder. The given acid supplements refer to 1 kg fodder. After two weeks, the rats were decapitated under anesthesia and the copper content of the liver determined by inverse polarography. The test results were evaluated by mathematical-statistical methods. ± values represent the standard deviation of the single

values.

Results and discussion

The test results are shown in table 1. The copper content of the liver of the control group which had been kept on a copper depletion diet was 7.5 ± 0.9 µg. The addition of $CuSO_4$ without simultaneous addition of the respective acids to the diet gave 24.2 ± 4.8 µg Cu. All other groups also showed a significantly higher Cu content of the liver.

Table 1. Total Cu content of the liver in µg.

	$CuSO_4$ plus 25 mg	250 mg of acid
Gluconic acid	24.3 ± 1.6	27.1 ± 6.4
Citric acid	25.3 ± 4.7	19.5 ± 4.6
Salicylic acid	22.7 ± 4.5	21.5 ± 2.9
Oxalic acid	22.4 ± 6.9	18.8 ± 6.1

After the addition of 25 mg of the acids the median values for the Cu content of the liver were nearly equal compared to the addition of $CuSO_4$ by itself. It can be presumed that the respective acids do not react with copper in this small supplement. The excess cations of the diet may have caught the ligands earlier.

When the supplements of the respective acids were increased to 250 mg per kg of fodder, the amount of copper deposited in the liver was, with the exception of gluconic acid addition, lower than in the comparative $CuSO_4$ supplement. In the case of oxalic and citric acid, the difference was significant. Several factors may have played a role in the decreased copper content of the liver. E.g., it is possible that these acids in high concentration function as enzyme inhibitors or have a direct influence on the naimal membrane which may have altered the total conditions of absorption.

Summary.

Copper absorption studies on rats were performed employing dietary administrations of gluconic, citric, salicylic, and oxalic acid respectively. Concentrations of 25 mg/kg diet of these acids had no effect, while a 250 mg supplement of citric or oxalic acid diminished the Cu absorption.

Translated by Carl Demrick Associates, Inc./CG/db

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Aus dem Institut für Tierernährung der TH München in Freising-Weihenstephan

Cu-Absorption bei Zulage von Glucon-, Citronen-, Salicyl- und Oxalsäure

7. Mitteilung

Zur Dynamik der Kupferabsorption

Von M. KIRCHGESSNER, U. WESER und H. L. MÜLLER

In früheren Arbeiten wurde die Absorption von komplexgebundenem Kupfer untersucht. Dabei zeigte sich, daß die Cu-Absorptionsrate von der Komplexgröße, der Komplexstabilität sowie vom spezifischen Absorptionsverhalten des Komplexliganden abhängt (KIRCHGESSNER und WESER, 1965a; KIRCHGESSNER et al., 1967). In der vorliegenden Arbeit soll geprüft werden, inwieweit die Absorption des Kupfers beeinflußt wird, wenn der Diät freie Säuren zugelegt werden. Hierzu wurden verschiedene N-freie organische Säuren ausgewählt, die in bestimmten Futter- bzw. Nahrungsstoffen auch in höherer Konzentration vorkommen.

Experimentelles

50 weiße weibliche Sprague-Dawley-Ratten mit durchschnittlichem Körpergewicht von 37 g wurden in Glas-Kunststoffkäfigen 17 Tage lang mit einer Cu-Mangeldiät depletiert (KIRCHGESSNER und WESER, 1965b). Anschließend wurden die Tiere in folgende zehn Gruppen aufgeteilt:

Keine Cu-Zulage
Cu-Sulfat

$CuSO_4$ + 25 mg Gluconsäure	$CuSO_4$ + 250 mg Gluconsäure
$CuSO_4$ + 25 mg Citronensäure	$CuSO_4$ + 250 mg Citronensäure
$CuSO_4$ + 25 mg Salicylsäure	$CuSO_4$ + 250 mg Salicylsäure
$CuSO_4$ + 25 mg Oxalsäure	$CuSO_4$ + 250 mg Oxalsäure

Die Cu-Konzentration betrug stets 50 µmol je kg Futter. Die angegebenen Zulagen an Säuren beziehen sich auf 1 kg Futter. Nach zwei Wochen wurden die Ratten unter Narkose dekapitiert und der Cu-Gehalt der Leber inverspolarographisch bestimmt (WESER und KIRCHGESSNER, 1964). Die Versuchsergebnisse wurden mathematisch-statistisch ausgewertet (LINDER, 1960), ±-Werte stellen die Standardabweichung der Einzelwerte dar.

Ergebnisse und Diskussion

Die Versuchsergebnisse sind in Tabelle 1 aufgezeigt. Bei der Kontrollgruppe, die auf Cu-Depletionsdiät gehalten wurde, betrug der Cu-Gehalt der Leber 7,5 ± 0,9 µg. Die Zulage von $CuSO_4$ ohne gleichzeitige Zulage der jeweiligen Säuren zur Diät ergab

Tabelle 1

Gesamt-Cu-Gehalt der Leber in µg

	$CuSO_4$ plus Säure	
	25 mg	250 mg
Gluconsäure	24,3 ± 1,6	27,1 ± 6,4
Citronensäure	25,3 ± 4,7	19,5 ± 4,6
Salicylsäure	22,7 ± 4,5	21,5 ± 2,9
Oxalsäure	22,4 ± 6,9	18,8 ± 6,1

24,2 ± 4,8 µg Cu. Ebenso zeigten alle anderen Gruppen einen signifikant höheren Cu-Gehalt der Leber.

Bei Zulage von 25 mg dieser Säuren lagen die Mittelwerte der Cu-Gehalte der Leber im Vergleich zur alleinigen $CuSO_4$-Zulage annähernd gleich. Es ist anzunehmen, daß die jeweiligen Säuren bei dieser geringen Zulage nicht mit Kupfer reagierten. Die überschüssigen Kationen der Diät dürften die Liganden vorher abgefangen haben.

Wenn die Zulage an den jeweiligen Säuren auf 250 mg je kg Futter erhöht wurde, so lagen mit Ausnahme der Gluconsäurezulage die in der Leber gespeicherten Kupfermengen vergleichsweise zur $CuSO_4$-Zulage niedriger; bei Oxal- und Citronensäure signifikant. Für diese verminderten Cu-Gehalte in der Leber können mehrere Faktoren eine Rolle gespielt haben. So ist z. B. denkbar, daß diese Säuren in hoher Konzentration als Enzyminhibitoren fungierten oder auf die tierische Membran selbst einwirkten, was insgesamt die Absorptionsverhältnisse geändert haben konnte.

Diese Untersuchung wurde mit Unterstützung der Deutschen Forschungsgemeinschaft durchgeführt.

Zusammenfassung

In Versuchen an Ratten wurde geprüft, inwieweit die Cu-Absorption durch Zulage von Glucon-, Citronen-, Salicyl- oder Oxalsäure zur Diät beeinflußt wird. Während die Cu-Absorptionsrate bei Zulage von 25 mg dieser Säuren je kg Futter nicht verändert wurde, war sie bei Zulage von 250 mg Citronen- oder Oxalsäure vermindert.

Summary

Cu-absorption studies on rats were performed employing dietary administrations of gluconic-, citric-, salicylic-, and oxalic acid, respectively. Concentrations of 25 mg/kg

diet of these acids had no effect while a 250 mg supplement of citric-, and oxalic acid diminished the rate of Cu-absorption, respectively.

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91. *New Copper Compounds Inducing Ovulation by Sex-Center Activation*

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(Comm. by T. KUMAGAI, M.J.A., June 13, 1960)

It is well known that the diencephalon controls incretion of gonadotropins from the anterior lobe of the pituitary gland and that gonadotropins stimulate sex-hormone production as well as ovulation of the ovary. Maldevelopment of the sex-center of women reveals two types of symptoms. The one is anovulation and the other is dysfunction or defect of sex-hormone production. The latter symptom can be treated by sex-hormone administration, but the therapy of the former is more difficult. Gonadotropins are used for treatment of anovulation, but do not act on the sex-center. Up to now, dysfunction of the sex-center has been hard to treat. Ovulation of rabbits induced by copper acetate and by copper sulfate was reported in the literature (Fevold, 1936; Bischoff, 1938; Emmens, 1940; Brooks et al., 1940; Mori and Nagasaki, 1940; Kobayashi, 1940; Harris, 1941; Ducy and Bradbury, 1942; Ducy and Bradbury, 1944; Naito, 1947; Sawyer and Markee, 1950; Kobayashi and Kobayashi, 1951, 1952; Kobayashi, 1953, 1955; Tsuno, 1957),[1] but none of these compounds is suitable for clinical use because of their toxicity. Barbiturate, which acts on the central nerve system, inhibits the copper ovulation of rabbits.[2] The pituitary gland of the rat was incubated in vitro with the diencephalon extract of the same animal and the gonadotropin production was measured. Gonadotropin increased distinctly, when the diencephalon from the rat previously injected with a copper compound was used (Sugiyama, 1960).[3] These experiments reveal that copper ions act on the sex-center. Accordingly, we have searched substances activating the sex-center and used extracts from plant leaves, estrogen, adenosine triphosphate, Isoniazid derivatives, Butazolidin, Meratran, Perphenazine, and Atmulin in order to induce ovulation, as given in the previous report (Kushima et al., 1959).[4] But the results were not satisfactory.

It is well known that ovulation in rabbits can be expedited by intravenous injection of copper acetate and copper sulfate. With the aim of discovering some copper compound with lower toxicity and with effectiveness in inducing ovulation, we tested the following compound.

Materials and methods. Rabbits of from 2,000 to 3,000 g of body weight were confirmed by laparotomy not to be in ovulation. 5,000 i.u. of estradiol benzoate was intramuscularly injected once a day for two days and solution of a copper compound was intravenously injected through the auricular vein of the rabbit on the third day. Occurrence of ovulation of the rabbit was examined by laparotomy forty-eight hours after the injection.

500 ml of solution of 200 mg per day of the copper compound was intravenously and quite slowly drip-injected into a woman in our clinic. Her ovulation was confirmed by measurement of basal body temperature.

Results of the experiment. We carried out experiments with phenol-sulfonate, amino-acetate and salicylate of copper, copper chlorophyl, and albumin copper as shown in Table I, but failed in inducing ovulation.

Table I. Intravenous injection of copper compounds

Item	Concentration of solution	Injected dose (mg)	Number of cases	Vital cases	Cases ovulated
Cu. phenol-sulfonate	0.5 mMol	26 - 39	3	3	0
	"	52	3	3	0
	"	104-208	3	3	0
Cu. amino-acetate	0.5 mMol	10 - 20	4	4	0
	"	25 - 45	5	0	
Cu. salicylate	0.5 mMol	20	2	0	
Cu. chloro-phyl	1.0 per cent	10 - 60	6	6	0
	"	100-200	2	2	0
Cu. albumin	1.0 per cent	30-200	6	6	0
	"	300	1	0	

When 10 mg of copper gluconate was injected, none of the subjects showed induced ovulation, but when the doses were raised to 12 mg, ovulation was induced in one of the two subjects (Table II).

Table II. Intravenous injection of copper gluconate (1)

Rabbit No.	Body weight (g)	Concentration (%)	Injected vol (ml)	Dose (mg)	Vital or lethal	Ovulation Right	Ovulation Left
170	3,000	0.1	10	10	vital	−	−
171	2,700	0.5	2	10	"	−	−
172	3,000	0.5	2	10	"	−	−
173	2,500	0.5	2	10	"	−	−
174	2,980	0.5	2	10	"	−	−
175	2,100	0.3	4	12	"	+	+
176	2,950	0.3	4	12	"	−	−

Of the 10 rabbits subjected to intravenous injection of 15 mg each of copper gluconate, 8 showed induced ovulation, but none of them suffered from spasms or died by the injected (Table III).

Table III. Intravenous injection of copper gluconate (2)

Rabbit No.	Body weight (g)	Concentration (%)	Injected vol (ml)	Dose (mg)	Vital or lethal	Ovulation	
						Right	Left
178	2,350	0.3	5	15	vital	+	−
179	2,500	0.3	5	15	,,	+	+
180	2,980	0.3	5	15	,,	−	−
181	3,000	0.3	5	15	,,	+	−
182	2,600	0.5	3	15	,,	+	−
183	2,250	0.5	3	15	,,	+	−
184	2,800	0.5	3	15	,,	+	−
185	2,400	0.5	3	15	,,	+	+
186	2,600	0.5	3	15	,,	+	+
187	3,300	0.5	3	15	,,	−	−

The toxicity of copper gluconate was of such a level as causing the death of the rabbits on the next day following intravenous injection of more than 45 mg of it. The LD50 of this drug for mice is 60 mg/kg.

Copper gluconate was used for the treatment of patients suffering from anovulation, and no subjective side effect was observed in total doses under 200 mg.

Summary. Ovulatory effects of copper gluconate were firstly reported. Ovulation was successfully induced by intravenous administration with this compound, which showed the least toxicity among the known copper compounds.

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The use of serum in the test has been compared to that of plasma. The thyro-binding of a serum has consistently been less than that of its plasma but the relationship between the two has not been constant. The source of this difference has not been identified, but the addition of anticoagulants to serum has no effect on thyro-binding.

The use of l-thyroxin (T4) would have an advantage over that of 1-triiodothyronine because of its greater stability (11). For this reason the test has been done using T4 as well as T3 and the results compared. The thyro-binding of T4 by plasma at two hours has been less than that of T3, a finding in disagreement with observations made by other methods and at different time intervals (11). The results with T3 and T4 were otherwise comparable, and a good correlation was shown between the thyro-binding indices determined by the two tests on different samples of plasma.

SUMMARY

The use of a resin previously labeled with 1-triiodothyronine-I^{131} makes possible a very simple qualitative measurement of the thyro-binding power of plasma. In clinical use this has been a satisfactory measure of thyroid function. With this simplification, and particularly with the commercial preparation of labeled resin, an easily and quickly done in vitro test of thyroid function is available to any laboratory possessing satisfactory I^{131} counting equipment.

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Experimental Studies for Scintillation Scanning of the Pancreas[1,2,3]

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INTRODUCTION

Since the pancreas has not been visualized radiographically without operative procedures, scintillation scanning should be studied as a method of mapping this major organ. Our initial efforts on the development of isotopic tracers to make scanning of the gland possible have given a moderate differential concentration in the pancreas and are reported briefly here.

Radioiodinated derivatives of organic acids and radiozinc chelates of amino acids have been studied in particular. The radioiodinated compounds were uniformly unsatisfactory because of high concentrations in the stomach and low values in the pancreas. Radiozinc chelates with glycine and arginine showed the greatest promise of the amino acid complexes. Their differential uptake by the pancreas was increased by the administration of other, stable, zinc salts, presumably by increasing the zinc content of the liver so that more radiozinc was eliminated by way of the pancreas.

METHOD

Male, Sprague-Dawley rats, weighing 400-425 grams, and fasted 18 hours prior to injection, were used for screening experiments. The radiozinc chelates were prepared by adding 10 ml of a normal saline solution of $Zn^{65}Cl_2$, containing approximately 1μc/ml, to 10 mg portions, respectively, of each of the 20 natural amino acids, or amino acid hydrochlorides. By the addition of 10 mg of anhydrous sodium acetate the reaction of the solutions was adjusted to, or near, pH 4. Control solutions were prepared by adding only the 10 mg of anhydrous sodium acetate to 10 ml of the $Zn^{65}Cl_2$ solution, thus forming $Zn^{65}(OAc)_2$. The tracers were given intravenously by tail vein, and the rats killed by exsanguination 1-8 hours later. The pancreas, liver, stomach, kidney, and spleen were dissected free of fat, and the activities per gram determined in a well counter.

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RESULTS

The highest concentration of activity in the pancreas after administration of an amino acid chelate alone was obtained with glycine zinc. The concentration of radiozinc in the pancreas was further enhanced by simultaneous injection of stable zinc gluconate, and slightly increased by stable zinc acetate. The data given in Table 1 are expressed as pancreas to liver ratios, P/L, obtained by dividing the cpm/gm of the pancreas by the count of the liver. Ratios for other competing organs are not given since in no case was the radioactivity of the other organs sufficiently high to interfere with the pancreas.

The highest P/L ratio obtained was 3.6, and this resulted 3 hours after 5 mg of zinc gluconate was given simultaneously with the glycine radiozinc. Injection of the same mixture to fed rats resulted in a P/L ratio of 3.2 at 2 hours. The highest ratio of the control radiozinc acetate was 1.1 at 1 hour.

DISCUSSION

An understanding of the clearance of zinc is essential for devising methods of increasing the uptake of radiozinc by the pancreas. Unfortunately, little clearance data are now available for the development of working hypotheses. In the single publication relating to zinc clearance, Gilbert and Taylor (4) imply that glycine zinc is cleared very rapidly from the blood but give neither specific pathways nor rates. To measure the overall physiological rate of clearance, stable serum protein zinc was exchanged with radiozinc in vitro by these authors, the product reinjected into rats, and in 30 minutes 95 per cent of the radiozinc was cleared from the blood.

TABLE 1. RATIO OF PANCREAS/LIVER (P/L) CONCENTRATIONS OF ZINC 65 GIVEN INTRAVENOUSLY UNDER VARIOUS CONDITIONS TO FASTED ADULT MALE RATS.

Tracer	*Added Zinc Gluconate (mg)*	*Hours after Injection* 1	2	3	4
Zinc—65 Acetate	0	1.1	0.7	—	—
Zinc—65 Acetate	2.5	1.4	2.5	1.5	—
Zinc—65 Acetate	5.0	—	1.8	2.2	2.8
Glycine Zinc—65	0	0.8	1.2	2.0	2.1
Glycine Zinc—65	5	—	2.8	3.6	1.6
Glycine Zinc—65	5 (fed)	—	3.2	1.9	1.9

Zinc is excreted from the body largely by way of the gastrointestinal tract. Although 10-20 per cent of zinc may be eliminated through the skin and hair, urinary and biliary excretions are very low. Experimental studies on the amounts excreted in the gastrointestinal tract, largely by way of the pancreas, vary widely. In zinc-65 studies, Sheline, Chaikoff et al. (6) found 50 per cent eliminated in 7 days in mice, and 23 per cent in 15 days in dogs. Gilbert and Taylor (4) determined with rats that 50 per cent of injected radiozinc was eliminated in 7 days by way of the gastrointestinal tract. As was shown by Birnstingl (3), injected zinc-65 appeared in pancreatic juice as early as 15 minutes after intravenous administration, and reached peak levels at 3-6 hours in dogs. The comprehensive studies of the metabolism of zinc-65 in rats by Ballou (1) show that the activity of the pancreas is moderately higher than liver, spleen and kidney three hours after intravenous injection under ether anesthesia.

The role of the liver is of key importance in zinc distribution. A variable but large portion of administered radiozinc in rats is taken up by the liver, and this is redistributed over a period of days with slow, steady incorporation in bones, skin, hair, and muscle (2). The reported values for the uptake by the liver vary. Thus, Meschan (5) found 85-88 per cent of injected zinc-65 in dog liver 2 hours after administration, and Sheline, Chaikoff et al., (7) reported 38 per cent at 3 hours. Since the liver is anatomically superimposed over the head of the pancreas, the high liver uptake of radiozinc creates an obstacle to effective scanning of the pancreas. Prevention of high level tracer zinc deposition in the liver is essential if the pancreas is to be scanned and this has been achieved in this study by simultaneously injecting stable zinc gluconate. With effective blockade of the binding sites of zinc in the liver, the level of radiozinc in the pancreas was increased markedly from a P/L ratio of 2.1 to 3.6. This approach offers promise for successful pancreatic scanning, but it will be necessary to increase the P/L ratio to 6 or more before clinical scanning can be considered.

SUMMARY

Radiozinc chelates with each of the naturally occurring amino acids have been examined as tracers for scintillation scanning of the pancreas. The studies have been made by tissue counts following intravenous administration of the chelates to fasted, male, adult rats. Glycine radiozinc accumulates most successfully in the pancreas to give radiozinc concentrations twice those found in the liver. Simultaneous administration of stable zinc gluconate with the glycine radiozinc increases the pancreas concentration to 3.6 times that of the liver.

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A STUDY OF SOME METAL GLUCONATES

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Summary

The properties of a series of metal gluconates isolated from acid and alkaline solution are described. The compounds examined were the simple gluconates of manganese(II), iron(II), zinc(II), cadmium(II), barium(II), and lead(II), and the complex hydroxy species $Cd_3(C_6H_{11}O_7)_2(OH)_4,2H_2O$, $Pb(C_6H_{10}O_7)_2(OH)_2$, and $Zn_2(C_6H_{10}O_7)(OH)_3$.

Introduction

Investigations of the solution chemistry of metal gluconate systems (cf. review by Sawyer[1]) indicate that each metal ion tends to behave in its own characteristic manner and the few solid systems which have been studied tend to confirm this generalization.

The structures proposed for isolated solid complexes of chromium(III) and aluminium(III) include compounds in which three gluconate ions are joined by two metal hydroxy species.[2] Similar stoicheiometry is reported for a thorium compound[3] together with another species having a metal : gluconate ratio of 3 : 4. In studies of the nickel gluconate system,[4,5] the solid hydroxy species from alkaline solution was found to possess a metal : gluconate ratio of 2 : 1.

The composition of the gluconate complexes formed by lead(II), cadmium(II), and zinc(II) in alkaline solution has now been investigated and the results are described in this paper. In addition, the properties of a number of simple metal gluconate salts, isolated from acid solutions, have been compared.

Experimental

Preparation of Metal Gluconates, $M(C_6H_{11}O_7)_2,nH_2O$

Metal ion (0·025 moles, carbonate salts of cadmium(II), lead(II), manganese(II), and barium(II), sulphide of iron(II), oxide of zinc(II)) were added to a hot aqueous solution containing 0·05 moles of glucono-δ-lactone. After boiling to hydrolyse the lactone and remove the gaseous reaction products, the solutions were filtered and cooled. Ethanol was added to the filtrate until

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[1] Sawyer, D. T., *Chem. Rev.*, 1964, 64, 633.

[2] Fialkov, Y. A., Grigoreva, V. V., Davidenko, N. K., and Pergshkina, N. G., *Farmatsevt. Zh., Kiev*, 1959, 14, 10 (*Chem. Abstr.*, 1959, 55, 7131*f*).

[3] Macarovich, C. G., and Czegledi, L., *Rev. roum. Chim.*, 1964, 9, 411 (*Chem. Abstr.*, 1964, 62, 4892*e*).

[4] Joyce, L. G., and Pickering, W. F., *Aust. J. Chem.*, 1965, 18, 783.

[5] Melson, G. A., and Pickering, W. F., *Aust. J. Chem.*, 1968, 21, 1205.

a precipitate was obtained. The solid was removed by filtration, washed with ethanol, dried over silica gel, ground to a fine powder and again dried.

Preparation of Metal Hydroxy Gluconates

The addition of a solution containing 0·05 mole of cadmium(II), zinc(II), or manganese(II) nitrate to a solution of 0·05 mole of sodium gluconate plus 0·075 mole sodium hydroxide resulted in the immediate precipitation of the hydroxy species. The moist pink manganese hydroxy species rapidly oxidized to brown manganese dioxide on contact with air.

The lead hydroxy compound dissolves in excess base, hence it was prepared by adding sodium hydroxide slowly to a lead gluconate solution (0·05M) until the pH reached 7·3. The white precipitate so obtained was allowed to stand in contact with the solution for 24 hr before being removed by filtration, washed with water, and dried over silica gel. Iron(II) and barium(II) did not yield any insoluble products under various conditions.

Instrumental Procedures

Thermogravimetric curves were obtained using a Stanton thermobalance with a heating rate of 100 deg/hr.

Infrared spectra (Nujol and hexachlorobutadiene mulls) were recorded on a Perkin–Elmer 125 and a Grubb–Parsons DM2 spectrophotometer.

Reflectance spectra were measured with a Perkin–Elmer 450 recording spectrophotometer and reflectance attachment.

The magnetic susceptibilities of the iron(II) and manganese(II) compounds at room temperature were measured by the Gouy method.

Melting points could not be ascertained, since decomposition generally preceded or accompanied melting.

Results and Discussion

The elementary analyses and empirical formulae of the species prepared are shown in Table 1. The number of water molecules of hydration were confirmed by the thermal curves (Fig. 1), although complete removal of the water was not complete in most cases below 175°. In the case of the salts of iron(II) and zinc(II), two of the associated moles of water were released more readily than the third. Decomposition of the gluconate skeleton begins at about 175° and ignition of the carbon char begins at 300°. The hydroxy compounds of lead and zinc appear to lose a mole of water at 200°, but this is considered to be due to either dehydration of a hydroxy bridge or selective decomposition of the gluconate skeleton.

Conversion of the simple metal gluconate into metal oxide was complete by 500°, the iron and manganese compounds requiring a lower temperature (400°). For the zinc compound, $Zn(C_6H_{11}O_7)_2,3H_2O$, the weight of residue at 550° was greater than required for oxide formation and corresponds to a product such as $5ZnO,2CO_2$ which is the product observed in the thermal decomposition of zinc carbonate.[6] Below 600°, the hydroxy compounds of lead and cadmium also failed to decompose to the simple oxide, the weight of residue in these cases suggesting the presence of metal hydroxide.

Two of the above compounds were coloured, the manganese salt being very pale pink and the iron salt yellowy green. Only the iron compound yielded a reflectance

[6] Duval, C., "Inorganic Thermogravimetric Analysis." 2nd Edn. (Elsevier: Amsterdam 1963.)

spectrum and this consisted solely of a very broad absorption band covering the region 25000–10000 cm^{-1}.

The observed effective magnetic moments (μ_{eff}) for the manganese(II) and iron(II) gluconates at 292°K were 5·89 B.M. and 5·21 B.M. respectively, characteristic of the metal ions in high-spin configurations.

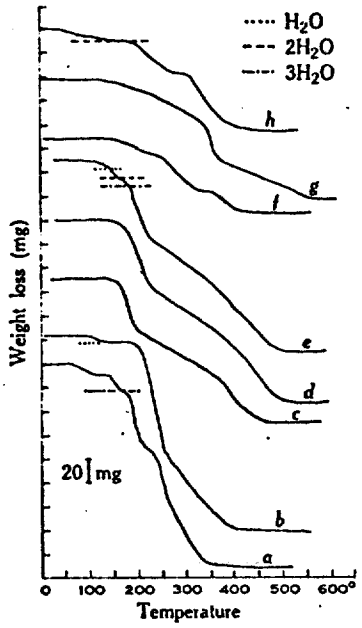

Fig. 1.—Thermogravimetric curves; 200-mg samples.
(*c*) $Fe(C_6H_{11}O_7)_2,3H_2O$;
(*b*) $Mn(C_6H_{11}O_7)_2,H_2O$;
(*c*) $Pb(C_6H_{11}O_7)_2$;
(*d*) $Cd(C_6H_{11}O_7)_2$;
(*e*) $Zn(C_6H_{11}O_7)_2,3H_2O$;
(*f*) $Pb_3(C_6H_{10}O_7)_2(OH)_2$;
(*g*) $Zn_2(C_6H_{10}O_7)(OH)_3$;
(*h*) $Cd_3(C_6H_{11}O_7)_2(OH)_4,2H_2O$.

TABLE 1

ANALYTICAL DATA FOR THE METAL GLUCONATES

Compound	Found			Calc.		
	C (%)	H (%)	M (%)	C (%)	H (%)	M (%)
$Fe(C_6H_{11}O_7)_2,3H_2O$	29·5	6·0	11·3	28·8	5·6	11·2
$Mn(C_6H_{11}O_7)_2,H_2O$	32·1	4·9	11·5	31·1	5·2	11·9
$Ba(C_6H_{11}O_7)_2,2H_2O$	25·0	4·5	24·4	25·6	4·6	24·4
$Cd(C_6H_{11}O_7)_2$	28·9	4·7	21·9	28·7	4·4	22·4
$Cd_3(C_6H_{11}O_7)_2(OH)_4,2H_2O$	17·3	3·1	40·5	17·4	3·4	40·7
$Pb(C_6H_{11}O_7)_2$	23·7	3·8	35·2	24·1	3·7	34·7
$Pb(C_6H_{10}O_7)_2(OH)_2$	13·7	2·1	60·5	13·9	2·1	59·6
$Zn(C_6H_{11}O_7)_2,3H_2O$	27·7	5·6	13·7	28·3	5·5	12·8
$Zn_2(C_6H_{10}O_7)(OH)_3$	19·6	3·5	34·8	19·2	3·5	34·9

The infrared spectra of all the compounds were similar and closely resemble those reported[5] for nickel gluconates and the sodium or calcium salts. Peak values in the individual spectra did not differ by more than ± 5 cm^{-1} from the following

typical spectrum (the bands in square brackets were not observed in the hydroxy compounds):

3300s(vb), 2970w, 2935w, 2875w, 2815w, 2760w(sh), 2710w(sh), 2670w(sh), 1585s(vb), [1465m], [1435m], 1390s, 1370m(sh), 1350m(sh), 1310m, 1265m, 1250m, 1225m, 1205w(sh), 1125m(sh), 1080s, 1055m, 1030s, 1005m(sh), 970w, 945w, 915w, 870w, 850w, 805m(sh), 780m, 715m, 685m, 645m, 590m, 560m, 510w, 440w.

Peaks appearing in the range 250–440 cm^{-1} have not been included in this list, since the spectrum in this region corresponded to that of water vapour. The broad absorption bands observed in the 3000–3400 cm^{-1} region (max. *c.* 3300 cm^{-1}) are also attributable to hydrogen-bonded water and/or alcohols. Since each gluconate skeleton contains five OH groups and is prone to the adsorption of small amounts of water, and since several of the salts were hydrated, infrared absorption in these regions merely confirmed the expected.

The broad absorption band at 1585 ± 5 cm^{-1} is assigned to the asymmetric $\nu(COO^-)$ with the symmetric stretch at 1390 ± 5 cm^{-1}. These bands are typical of the salts of carboxylic acids[7] and are accompanied by absorption peaks corresponding to bending modes of the carboxylate group near 800, 720, and 650 cm^{-1}. The bonding between the metal ion and this group is thus considered to be essentially electrostatic in all cases. However, in order to satisfy the usual coordination pattern of the various metal ions examined, some association of the metal ion with alcoholic groups must occur.

Primary and secondary alcoholic groups give rise to absorption bands, associated with O–H deformation and C–O stretching frequencies, in the region between 1000 and 1400 cm^{-1}.

In sodium gluconate, where interaction between the metal ion and alcoholic groups is assumed to be minimal, the absorption at 1090 cm^{-1} was assigned to a secondary hydroxyl group.[5] Comparison of this spectrum with those of the barium(II), manganese(II), iron(II), zinc(II), cadmium(II), and lead(II) compounds indicates a 5–15 cm^{-1} shift of this band to lower frequencies in the presence of the heavy metal ions. This shift is considered to confirm that there is some interaction between the metal ion and one or more of the secondary alcoholic groups.

Structural Considerations

The configuration of the gluconate ion in alkali metal salts has been elucidated by an X-ray study,[8] and while models based on this structure indicate that the zig-zag chain could twist sufficiently to allow the carboyxl group and two hydroxyl groups to occupy coordination positions around a metal ion, the similarity of the infrared spectra and the presence of water of hydration in some of the compounds indicates that marked distortion of the original gluconate structure is not favoured. In the simple gluconates, the hydroxy acid acts as a bidentate ligand.

It has been shown[8] that the C 2–O 2 bond (i.e. the α-hydroxy group) lies approximately in the plane of the carboxylate group, as in tartrates and tartaric acid, and the

[7] Nakamoto, K., "Infrared Spectra of Inorganic and Coordination Compounds." (John Wiley: New York 1963.)

[8] Littleton, C. D., *Acta crystallogr.*, 1953, 6, 775.

observed shift to lower frequencies of the 1090 cm^{-1} secondary alcohol peak is similar in the gluconate compounds to that observed in the tartaric acid, copper tartrate system. N.m.r. studies[1] of lead and bismuth gluconate compounds indicate that the gluconate residue is bonded by the α hydroxyl and carboxylate groups. This evidence suggests that the most probable second coordination site, for the series of gluconate compounds studied, is the α-hydroxy group. An X-ray study of lead gluconate [$Pb(C_6H_{11}O_7)_2$] indicated[9] that the two gluconate residues attached to the lead atom extend above and below along the *c* direction, the disposition of these residues necessitating a large cell (12 molecules per cell).

In the hydroxy compounds of lead, cadmium, and zinc, the OH bending modes which produce absorption peaks at around 1450 cm^{-1} were not observed. This suggests that some or all of the other alcoholic groups are coordinated, to yield a more rigid structure, and as the empirical formulae of these compounds include some hydroxy groups, it is highly probable that several gluconate residues are joined by hydroxy species as proposed for the aluminium, chromium, and thorium compounds.

[9] Popinsky, R., *Phys. Rev.* (*A*), 1942, **61**, 726.

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(German publication, pp. 286-302)

Blood sugar effect of zinc compounds

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Except for zinc-containing insulin preparations, little research has been done on the effect of zinc compounds on the carbohydrate metabilism. In 1892, Italian authors (1) reported on considerable glycosuria in dogs that were given 0.5-1 g of zinc with the food. In 1918, Salant and Wise (2) communicated that feeding or injection of zinc salts caused hyperglycemia and glucosuria in rabbits, dogs and cats. When zinc acetate was given by mouth, rabbits showed glucosuria and albuminuria at 335 mg of zinc/kg but not yet at 30-100 mg Zn/kg. Subcutaneously given zinc malate in doses of 50-100 mg Zn/kg brought about glucosuria and albuminuria, while intravenously a slight glucosuria could still be produced with doses of 9-10 mg Zn/kg as zinc malate, resulting in blood sugar values usually ranging about 200 mg%. Nearly all animals showed albuminuria and died in the course of 2-9 days after zinc intake. Cats reacted with glucosuria after subcutaneous injection of 25-100 mg Zn/kg as zinc malate; dogs were given 15-26 mg Zn/kg as zinc malate, and glucosuria sually occurred. But these dogs survived the zinc injection by only 1-5 days.

The effect of smaller doses of injected zinc salts on the blood sugar of dogs was examined by Sanfilippo (3) (0.87 mg Zn/kg each i.m. and i.v. as zinc chloride, bromide, iodide, nitrate, sulfate, lactate and acetate). He found no alteration of the normal glucose level. Also Berenshtein and Shkolnik (4) observed no changes of the blood sugar in rabbits and dogs after subcutaneous injection of zinc sulfate or acetate in doses of 100-200 gamms Zn/kg but did so after higher doses (0.5-5.0 mg Zn/kg).

To summarize, these studies show that to produce hyperglycemia and glucosuria high doses of zinc are necessary. But these are for the most part highly toxic, so that the animals often perish in the acute stage of the test. When giving smaller doses of zinc, the glucosuria disappears, while the hyperglycemia persists at first but fails to occur if the dose is reduced further. In the case of parenteral intake, the literature would indicate that the dose while still causes a distinct hyperglycemia is in the range of about 1-5 mg Zn/kg. It is not evident from the cited research, however, whether the degree of the zinc effect is varied by the chemical structure of the zinc compound supplied or whether its composition is of no importance for the hyperglycemizing effect.

We examined the effect of different acid radicals combined with zinc as to the degree and duration of the zinc hyperglycemia; in particular we investigated whether it is possible with suitable organic radicals, e.g. with complex zinc compounds, to bring about a blood sugar increase using small doses of zinc, that is, whether doses of as little as 1 mg Zn/kg and down to fractions of one gamma Zn/kg can still influence the blood sugar.

The present paper deals with -

(1) zinc salts of nitrogen-free acids which were given parenterally to rabbits in doses of 1 mg down to 0.001 gamma Zn/kg. The following zinc salts were tested:

Chloride	Pyrophosphate	Malate	Gluconate
Sulfate	Citrate	Maleinate	Glucuronate
Acetate	Tartrate	Pyruvate	Ascorbinate

(2) zinc salts of alpha-amino acids, in which the stability of the coordinate zinc bond can be modified widely according to the kind and number of the amino acid radicals. No data were found in the literature on the effect of zinc amino acid complexes on the blood sugar; we tested the oral, intra-

muscular and intravenous intake of zinc glycocoll, zinc analine, and zinc glutaminate, using doses of several mg Zn/kg down to 0.001 gamma Zn/kg.

Method and substances

All experiments were on rabbits kept without food for 24 hours. Dissolution of the zinc compounds to be injected in 0.9% NaCl solution, adjustment of the pH to 6.4 - 7.0. Only freshly prepared solutions were used. Blood sugar tests according to Hagedorn-Jensen or according to Fujita and Iwatake (5). The number and time intervals of the blood samplings are evident from the figures.

The zinc compounds to be tested were prepared in solid form, with the exception of zinc chloride, sulfate and acetate (commercial products p.a.) and zinc pyrophosphate (see below). The zinc content was determined by direct micro-titration with ethylene diamine-tetraacetic acid as disodium salt (Complexon III) according to Flaschka (6). Indicator: Eriochrome Black T. Calculation of the quantity of substance required for the animal test on the basis of the zinc analysis (*).

The zinc salts were obtained by reaction of the respective acid dissolved in water with the calculated quantity of zinc carbonate, possibly with precipitation of the resulting zinc salt by ethanol. Instead of the very little soluble $Zn_2P_2O_7$, which is unsuitable for purposes of injection, we used an acid zinc pyrophosphate, which was obtained in dissolved form from $ZnCO_3$ and aqueous pyrophosphoric acid. In the same manner zinc citrate and zinc malate were prepared, precipitating them from the aqueous solution by adding the same volume of ethanol. Zinc tartrate, zinc maleinate and zinc pyruvate precipitated spontaneously from water as amorphous products; these three salts could be used only in doses of at most 100 gamma and 10 gamma Zn/kg (see below) because of their low solubility. Zinc gluconate obtained from gluconic acid lactone and $ZnCO_3$, formed a granular crystalline precipitate and had the composition stated in the literature (7): Zn (gluconate)$_2$ + $5H_2O$. In preparing

zinc glucuronate from glucuronic acid lactone and $ZnCO_3$, we noted that in order to obtain a zinc salt which will not immediately decompose by hydrolysis at neutral pH, it was necessary to take an excess of glucuronic acid (Zn : glucuronic acid = 1 : 3.5). We then obtained the salt in yellow crystals from 50% ethanol. Zinc ascorbinate was made available to us by courtesy of Hoffmann-La Roche, Basel, in the form of a yellow amorphous powder having a zinc content of 18.3%.

Glycocoll, alanine and glutaminic acid give, depending on the manufacturing method, zinc salts of different composition which are clear when dissolved in water in neutral reaction or which may be decomposed more or less quickly by water with formation of flaky precipitates. Zinc-amino acid complexes, which in aqueous solution at neutral pH become cloudy very soon after their dissolution and release zinc hydroxide, are unsuitable for injecting. In our tests we used only those zinc-amino acid complexes whose stability to water was great enough to avoid decomposition at neutral or weakly alkaline pH. Among them, however, the differences of action are great; few zinc complexes are able to cause great increases in blood sugar at doses under 100 gamma Zn/kg. Unless otherwise indicated, the test results discussed below on zinc amino acids relate to highly active complexes.

Results

1. Zinc salts of N-free acids

Zinc chloride and zinc sulfate

The intravenous injection of $ZnCl_2$ in doses of 1 mg, 100 gamma, 10 gamma, 1 gamma and 0.01 gamma Zn/kg did not lead to any appreciable blood sugar increases; only once, after 1 mg Zn/kg, a blood sugar increase from 112 to 132 mg% occurred following the injection. But two other animals did not show this effect after the same dose. The same thing occurred with $ZnSO_4$. In one animal 1 mg Zn/kg produced definite hyperglycemia immediately following the injection, with a peak of 164 mg% after 25 min, while a second animal

reacted to the same dose with normal values. Lower doses of zinc down to 0.01 gamma Zn/kg (as with $ZnCl_2$) showed no clear and reproducible blood sugar-increasing effects.

In agreement with the literature (2, 3, 4) 1 mg Zn/kg evidently represents the lower limit of the hyperglycemizing dose for $ZnCl_2$ and $ZnSO_4$. Doses under 1 mg Zn/kg no longer show a distinct effect, although occasionally a slight blood sugar increase can still be observed even at very small doses (e.g. 1 gamma Zn/kg). What is remarkable is that the animals tolerate the intravenous injection of these highly ionized zinc salts without acute symptoms; also subsequent damage was not observable.

Zinc acetate

Compared with $ZnCl_2$ and $ZnSO_4$, the picture of the blood sugar curves after zinc acetate i.v. changes inasmuch as slight blood sugar increases occur somewhat more frequently, though not always reproducibly (usually 20-30 mg%). These increases, however, are not dependent on the dose, for even after a few gamma or as little as 0.001 gamma Zn/kg as zinc acetate i.v. the blood sugar is seen to increase. The curves given in Fig. 1 for 0.01 gamma and for 0.001 gamma Zn/kg i.v. may serve as an example.

Fig. 1 0.01 gamma Zn as zinc acetate i.v. (solid line) K 288; 0.001 gamma Zn as zinc acetate i.v. (broken line) K 298.

Zinc pyrophosphate

In the experiments with acid zinc pyrophosphate at doses of 1 mg Zn/kg to 0.01 gamma Zn/kg we saw in no case (17 animals were tested) blood sugar increases in excess of the normal fluctuations.

Zinc citrate

In comparison with $ZnCl_2$, $ZnSO_4$ and zinc acetate, we find no blood sugar increasing effect with zinc citrate. On the basis of 14 rabbit tests, we have

the impression, instead, that zinc citrate influences the blood sugar only in exceptional cases, and then not at all dependent on the dose, as there was a marked lack of response precisely with doses of 1 mg and 100 gamma Zn/kg.

Zinc tartrate

Zinc tartrate, which for reasons of its solubility could be tested only in doses of 10 gamma, 1 gamma and 0.01 gamma Zn/kg, showed the same behavior as zinc citrate.

Zinc malate

The intravenous injection of zinc malate (18 tests on rabbits) led to blood sugar increases at all doses tested in about 50 per cent of the cases. We give as examples in Fig. 2 blood sugar curves as obtained after injection of 1 mg and 1 gamma Zn/kg as zinc malate.

Fig. 2 1 mg zinc/kg as zinc malate (solid line) K 249; 1 gamma zinc/kg as zinc malate (broken line) K 248.

Zinc maleinate

With zinc maleinate, because this salt is little soluble, we did not try the dose of 1 mg Zn/kg in order to avoid the injection of large volumes. In most cases the doses of 100 gamma to 0.01 gamma Zn/kg led to initial blood sugar increases which, however, were so slight that we could not be sure that they are attributable to the injected zinc complex.

Zinc pyruvate

Again, because this compound is little soluble, it was not possible to bring the dose of 1 mg Zn/kg into a volume suitable for injection. Intravenous injection at doses of 100 gamma, 10 gamma, 1 gamma and 0.01 gamma Zn/kg caused no ratable effect on the blood sugar in 12 rabbits.

Zinc gluconate

After injection of zinc gluconate (14 rabbits) distinct blood sugar increases occurred rarely. The remarkable thing is that precisely the smallest doses (0.01 gamma Zn/kg) caused the most marked blood sugar increases; cf. Fig. 3.

Fig. 3 Doses of 0.01 gamma Zn/kg i.v. each as zinc gluconate. K 278 (solid line) and K 315 (broken line).

Zinc glucuronate

Has about the same effect as zinc gluconate (14 rabbits tested). As examples we give in Fig. 4 two curves showing the blood sugar response after injection of 1 gamma Zn/kg as zinc glucuronate.

Fig. 4 1 gamma Zn/kg i.v. each, as zinc glucuronate. K 242 (solid line) and K 272 (broken line)

Zinc ascorbinate

The same result as with zinc gluconate and glucuronate was obtained with zinc ascorbinate in 24 tests on rabbits. The blood sugar increases were in part questionable, in part unambiguous, but independent of the dose. As examples we give inFig. 5 the blood sugar response after injection of 100 gamma Zn/kg and 0.01 gamma Zn/kg as zinc ascorbinate.

Fig. 5 100 gamma Zn/kg i.v. as zinc ascorbinate. K 324 (solid line); 0.01 gamma Zn/kg i.v. as zinc ascorbinate, K 241 (broken line).

Control tests

With those of the above named zinc complexes which proved to affect the blood sugar, control tests were carried out with the respective free acids or their salts. Sodium malate, glucuronate and ascorbinate as well as free ascorbic acid did not increase the blood sugar at the doses in question. Only with sodium gluconate initial blood sugar increases were encountered in the range from 8 mg down to 8 gamma gluconic acid/kg, but not at corresponding doses of calcium gluconate.

2. Zinc salts of alpha-amino acids

In the following, the test results are arranged in three groups according to the quantity of zinc supplied per kg of body weight:

A. More than 1 mg Zn/kg; B. 1 mg to 1 gamma Zn/kg; C. Less than 1 gamma Zn/kg.

A. Doses over 1 mg Zn/kg

Fig. 6 shows the blood sugar response after oral administration of zinc glycocoll and zinc glutaminate (14 and 10 mg Zn/kg, respectively). During the first 10 hours after intake of the zinc compound, considerable hyperglycemia occurs, accompanied by glucosuria and albuminura. Also during the following days, hyperglycemic states were observed again and again, interrupted by normal and also subnormal glycemia values. A slight glucosuria could be demonstrated still on the fourth day after zinc had been fed. Other tests with zinc glycocoll and zinc glutaminate showed the same pattern as the curves shown in Fig. 1, which are typical of dysregulation of the blood sugar in zinc poisoning. The doses used are, in fact, highly toxic; all animals treated with them perished after 2-10 days.

Fig. 6 14 mg Zn/kg oral as zinc glycocoll, K 60 (solid line); 10 mg Zn/kg oral as zinc glutaminate, K 66 (broken line).

Salant and Wise (2) needed 335 mg Zn/kg oral as zinc acetate to produce glucosuria in the rabbit; with 30-100 mg Zn/kg this was not possible. With zinc-amino acid complexes glucosuria can be produced with much smaller doses of zinc, as can be seen from Fig. 6. This is no doubt due mostly to the fact

that the zinc-amino acid complexes used are resorbed very well, unlike zinc acetate. From stable zinc-glycocoll complexes for example, hardly any zinc ions are released in the gastro-intestinal tract; the typical heavy metal effects do not occur. For example, a zinc glycocoll soluble as a clear solution at neutral pH causes neither a metal taste nor nausea or vomiting in man after oral intake.

Fig. 7 2.5 mg Zn/kg i.m. as zinc alanine, K 207 (solid line) and K 295 (broken line).

In Fig. 7 are shown two blood sugar curves after intramuscular supply of 2.5 mg Zn/kg as zinc alanine. The beginning blood sugar increase is demonstrable already 15 minutes after the injection; the hyperglycemia lasts 4-5 hours. In one animal (K 207) the blood sugar curve leads to slightly hyperglycemia values again after the injection. These animals survived.

The intramuscular supply of amino acid-zinc complexes at higher doses, e.g. 28 mg Zn/kg as zinc glycocoll or 20 mg Zn/kg as zinc glutaminate, had the same effect on blood sugar and general condition of the animals as described above under Fig. 6 for the oral doses; 5 out of 6 animals perished after 3-5 days.

Fig. 8 6 mg Zn/kg intravenous as zinc glutaminate, K 65 (solid line); 8.5 mg Zn/kg intravenous as zinc glycocoll, K 62 (broken line).

Fig. 8 shows the response of the blood sugar curves after intravenous injection of several mg of Zn as zinc glycocoll (8.5 mg Zn/kg) and zinc glutaminate (6 mg Zn/kg). In both cases one observes hyperglycemic values, also glucosuria and albuminura, as long as 80 hours after the injection, with blood sugar decreases to about 50 mg% occurring in between. Intravenous supply of more than 5 mg Zn/kg in the form of inner zinc complexes of alpha-amino

acids caused death in 7 out of 8 animals. K 62 in Fig. 8 succombed after 80 hours; in parallel tests with the same dose the animals perished 60 and 100 hours after the injection. K 65 (see Fig. 8; 6 mg Zn/kg) was the only one to survive the zinc load.

With intravenous injection of 5 to 1 mg Zn/kg the degree and duration of the hyperglycemia became less; at the same time the toxicity decreased greatly, 4 out of 5 animals surviving.

Fig. 9 1 mg Zn/kg oral as zinc glycocoll, K 293 (solid line); 50 gamma Zn/kg i.m. as zinc glycocoll, K 288 (broken line)

B. Doses of 1 mg to 1 gamma zinc/kg

As can be seen from Fig. 9, an intramuscular dose of as little as 50 gamma Zn/kg as zinc glycocoll causes a pronounced hyperglycemia lasting several hours. Given orally, also 1 mg Zn/kg as zinc glycocoll has a definite effect. The least effective oral dose was not tested because of the undefined resorption conditions of rabbit intestine.

Fig. 10 850 gamma Zn/kg i.v. as zinc glycocoll, K 76 (solid line); 141 gamma Zn/kg i.v. as zinc glycocoll, K 90 (broken line).

Fig. 11 14.1 gamma Zn/kg i.v. as zinc glycocoll, K 78 (broken line); 3.5 gamma Zn/kg i.v. as zinc glycocoll, K 96 (solid line).

Figures 10 and 11 show blood sugar curves after intravenous injection of zinc glycocoll in the range from 850 gamma to 3.5 gamma Zn/kg. Also these doses still cause considerable hyperglycemia demonstrable as early as 15 min after the injection, and which may attain values between 160 and 200 mg% within the first hour. Generally the blood sugar increases still persist several hours after the injection.

Fig. 12 0.5 gamma Zn/kg i.m. as zinc glycocoll, K 246 (brokenline); 0.5 gamma Zn/kg i.v. as zinc glycocoll, K 296 (solid line).

Fig. 13 0.01 gamma Zn/kg i.v. as zinc glycocoll, K 276 (solid line); 0.001 gamma Zn/kg i.v. as zinc glycocoll, K 203 (broken line).

C. Smaller doses than 1 gamma Zn/kg

In figures 12 and 13 are shown tests with parenteral supply of less than 1 gamma Zn/kg. As the doses decreases to every smaller quantities of zinc, the degree and duration of the blood sugar increase diminish; the hyperglycemia manifests itself as a steep blood sugar increase of short duration, nearly always occurring immediately after the injection and often followed by a second, smaller fluctuation.

The initial hyperglycemia after these surprisingly small doses of zinc occurs more or less regularly, depending on the type of zinc-glycin complex given, almost without exception with the most suitable zinc-glycin complex (70 tests on rabbits with doses below 1 gamma Zn/kg as zinc glycocoll). The least effective dose was not determined, but a random test with 0.0001 gamma Zn/kg of the most effective zinc-glycin complex still produced a distinct blood sugar increase.

Discussion of the results

1. Zinc salts of N-free acids

In comparison with the literature references mentioned above (1, 2, 4) on hyperglycemia and glucosuria after intake of high and toxic quantities of zinc, the present studies show that zinc salts of nitrogen-free acids in doses of 1 mg Zn/kg i.v.and less are able to produce blood sugar increases which, however. do not occur regularly. What is remarkable is that minute doses of 1 gamma or 0.01 gamma Zn/kg, for example, can still cause an initial rise of the blood sugar, and that in the range from 1 mg to 0.01 gamma Zn/kg i.v.

there is no dependence on the dose. On the basis of control tests with the free acids or their sodium salts, the blood sugar-increasing effect must be ascribed to the zinc.

The differences in action between inorganic and organic zinc salts or between highly complex, slightly complex and non-complex zinc salts are not marked enough to permit reliable conclusions as to the possible relationships between the type of metal bond and the blood sugar-increasing effect. Yet the curves convey the impression that the structure of the acid radical combined with the metal is not indifferent for the effect of the zinc on the blood sugar. It seems to us expecially striking and biologically remarkable that very small doses, e.g. 1 gamma or 0.01 gamma Zn/kg, can influence the blood sugar level, as these are quantities which are within the physiological range and which in our experiments are nearly always attached to cell-related acid radicals.

The zinc doses given by us, which were not in excess of 1 mg Zn/kg, showed no toxicity whatever. However, Valles et al. (8) observed in the dog after intravenous injection of 4 mg/kg zinc gluconate paralysis of the kind legs, reduced tendon reflexes and general atony, while 2 mg/kg zinc gluconate was tolerated well by dogs as well as by man.

2. Zinc salts of alpha-amino acids

A survey of the findings made with zinc-amino acid complexes gives a much different picture as compared with the very weak action of the zinc salts of nitrogen-free acids.

If we consider first those of our experiments with zinc-amino acid complexes where more than 1 mg Zn/kg was given, we find in all cases a considerable hyperglycemia, and in part glucosuria. Yet, compared with the doses used by Salant and Wise (2) - 335 mg Zn/kg as acetate orally, 25-100 mg/kg as malate subcutaneously, 15-26 mg Zn/kg as malate intramuscularly and 9-10

mg Zn/kg as malate intravenously - the quantities administered in our experiments are much smaller; the lower limit of the American authors corresponds approximately to the upper limit of the zinc doses tested by us. The considerably higher action of the zinc-amino acid complexes compared with the zinc salts of nitrogen-acids must be attributed no doubt, in part, to the good resorption of these compounds when given orally, as has been mentioned before.

The masking of the heavy metal by a stable coordinate bond, as it exists in the zinc-amino acid complexes used, evidently also contributes to increased zinc effect when given parenterally. A variation of the zinc effect by the type of the amino acid radical was not observed by us at doses over 1 mg Zn/kg, if these were solid complexes which remained in solution and clear at neutral pH. Zinc glycocoll zinc alanine and zinc glutaminate in mg doses practically did not differ in their hyperglycemizing effect. Glucosuria was always observable only if hyperglycemia occurred also; a lowering of the kidney threshold for glucose could not be demonstrated.

While zinc-amino acid complexes in doses between 1 and 10 mg Zn/kg often cause a dysregulation of the blood sugar lasting several days, the test results vary in three respects when using less than 1 mg Zn/kg down to fractions of a gamma Zn/kg.

1. Degree and duration of the hyperglycemia decrease with decreasing zinc dose, but the blood sugar increase continues to occur following the injection. At less than 1 gamma Zn/kg the dependence on the dose is no longer detectable.

2. The toxicity decreases with decreasing dose.

3. Minor variations in the complex-chemical structure become noticeable together with the specificity of the amino acid radical.

Point 3 deserves to be noted especially, as it hints to relationships between specific complex structure and hyperglycemizing effect. The basic condition for zinc-amino acid compounds in gamma doses to bring about a blood sugar increase is, as has been mentioned before, that they must be complexes

which release no zinc hydroxide even if left standing for several days in aqueous solution at neutral pH. On the other hand, the testing of some zinc-amino acid complexes with an especially stable metal bond, such as zinc asparagine, zinc histidine, zinc histidyl histidine, zinc cysteine and zinc glutathione, proved that these compounds do not influence the blood sugar at doses under 1 mg Zn/kg. It is, however, possible to form stable zinc complexes also of glycocoll which at doses of some 100 gamma Zn/kg do not have a blood sugar increasing effect.

If we compare the zinc-amino acid complexes with the zinc salts of N-free acids (see above) with respect to their hyperglycemizing effect, we find the following: While the blood sugar increases after administration of zinc salts of N-free acids in doses of 1 mg Zn/kg and less are very small, much stronger and regularly occurring effects are found at the same doses of suitable zinc-amino acid complexes; besides, in the range from 10 mg Zn/kg down to about 1 gamma Zn/kg there is a dependence between dose and degree of effect. At doses of 1 gamma to 0.001 gamma Zn/kg, a gradation of the effect according to the amount of zinc supplied is no longer observable; evidently the effect is then influenced more strongly by individual differences in the respective metabolism of the test animals.

Control tests with amino acids: The literature (9, 10, 11) contains observations on hyperglycemizing effects of amino acids, although much higher doses are necessary to produce blood sugar increases than in our experiments. The control tests carried out by us with amino acids (testing of all amino acids, given also as zinc salts, in corresponding dosage) showedthat the blood sugar increases achieved with zinc-amino acid complexes are attributable to the complex-bound zinc. This is proven also by the fact mentioned above that from the same amino acid we have zinc complexes of different composition, some of which are effective at doses under 1 mg Zn/kg, while others do not influence

the blood sugar.

Toxicity: While in testing the zinc salts of N-free acids the doses (not higher than 1 mg Zn/kg) showed no harmful effect on the animals (see above), the zinc salts of amino acids given in quantities of more than 1 mg Zn/kg caused in many cases pronounced toxic symptoms, which were not entirely absent even at doses of 1 mg Zn/kg and less. Let us briefly summarize our observations on the toxicity of these compounds:

The general behavior of the test animals after injection of zinc-amino acid complexes depends on the size of the dose. Doses over 1 mg Zn/kg as zinc glycocoll or as zinc glutaminate led to an increasingly severe state of collapse, sometimes lasting several days, where it was difficult to draw blood from the cold ears. The animals sat crouching, their respiration was accelerated. About two thirds of the rabbits did not get out of this state and perished after 48 to 120 hours. In most of them paresis of the hind legs and in some cases also bladder paralysis had occurred. Doses of a few mg Zn/kg caused death only after 8-14 days in several cases, the animals being in a much reduced state of nutrition. Of the other rabbits only a small number tolerated these zinc doses without impairment of their general state, the rest recovered slowly and survived without evident late symptoms. The extent of the toxic phenomena at high doses was not dependent on the method of application - i.v., i.m. or by stomach probe.

Doses of 1 mg to about 20 gamma Zn/kg in the form of the above mentioned amino acid complexes caused a much lower degree of impairment of the circulation, in linear proportion to the dose. At reduced dose also the number of animals which showed no toxic symptoms increased. Some few animals died in a reduced state after 10-14 days.

Doses of under 20 gamma Zn/kg caused a toxic change of the general state only in exceptional cases. As such an exception we may cite the occurrence of

a complete atonic paralysis of the hind legs and paralysis of the bladder 15 minutes after injection of 0.35 gamma Zn/kg i.v. as zinc glycocoll (K 122).

For the comparable doses of 1 mg Zn/kg and less it follows that zinc salts of amino acids are generally more toxic than zinc salts of nitrogen-free acids.

Concerning the mechanism of action which causes the blood sugar increase by zinc compounds, nothing definite can as yet be said. Hints may perhaps be derived from older studies by Hausler and Schnetz (12): These authors examined on the isolated frog liver the effect of metals on normal glycogenolysis and glycogenolysis increased by adrenalin. They found that zinc (besides Cu and Hg) clearly increases the release of sugar from the frog liver in certain concentration ranges (10^{-4} to 10^{-6} millimole metal salt per liter in the perfusion liquid), while this was not the case at higher (10^{-2} to 10^{-3}) or lower (10^{-7} millimole $ZnSO_4$/liter) concentrations. But if adrenalin was added to the perfusion liquid together with the metal, the zinc completely stopped the increase in glycogenolysis caused by adrenalin in the control test. It follows from these experiments of Hausler and Schnetz that zinc in very low concentrations can be glycogenolytic at least on the isolated frog liver, this effect being brought about without the involvement of adrenalin.

In further tests, however, Schnetz (13) came to the conclusion that in the whole animal zinc, cadmium and copper salts clearly reduce the adrenalin hyperglycemia, and that the normal blood sugar level is "not substantially influenced by said metals." This is contrary to the findings of Berenshtein and Shkolnik (4), who observed an increase in the adrenalin hyperglycemia when $ZnSO_4$ was injected at the same time.

For the interpretation of the blood sugar increases observed by us after supplying very small doses of zinc it is logical to think of an effect of corresponding enzyme systems which contain zinc in the molecule or are activated by zinc. There enter into consideration, for example, phosphatase

activations, because generally phosphate transfer is increased by bivalent metals and zinc is said to be contained in the active group of phosphatases (14, 15, 16).

In view of the scanty and inconsistent data of the literature, we were anxious to provide a reliable experimental basis concerning the hyperglycemizing effect of zinc compounds and to explore moreover the previously unknown blood sugar increasing effects of very small zinc doses. The 13 figures contained in this paper are the result of about 500 tests on rabbits and are each characteristic of a relatively large test series. As has been shown above, no pronounced effects on the blood sugar level can be obtained with the zinc salts of 12 N-free acids in doses of 1 mg Zn/kg and less; in view of our numerous experiments we would regard this judgment as final. For the zinc-amino acid complexes, on the other hand, further intensive study is necessary, especially from a chemical point of view, since until now their action in gamma doses was as little known as the fact that zinc-amino acid complexes of the same amino acid but of different composition may respond differently biologically. The latter fact is not clearly evident at high doses (over 1 mg Zn/kg), while at smaller doses (under 1 mg Zn/kg) considerable differences of action are observable in the individual complexes. In another paper we shall report on the results we obtained in investigating the relationships between hyperglycemizing action and structure of the coordinate zinc bond.

Further investigation of these relationships should be especially interesting with regard to the biologic behavior of zinc-containing natural substances, e.g. hormones and enzymes, as the zinc-amino acid complexes constitute simple model substances which permit thepossible variations of the complex zinc bond to be relatively easy to observe and to prepare. Based on our test results, it is to be expected also in the case of zinc, as with other metals, that minor variations of the complex structure can have a high degree of influ-

ence on the behavior of zinc compounds in the metabolism. Consequently, the findings here reported on the blood sugar increasing effect of gamma doses of suitable zinc-amino acid complexes lead to the question whether nature, too, makes use of this blood sugar increasing principle. This seems to be the case, for meanwhile we have been able to show that hyperglycemizing extracts of pancreas and gastric mucosa regularly contain zinc in complex form, which in these extracts evidently participates in the blood sugar increasing action.

Summary

The following zinc salts were given intravenously to rabbits in doses of 1 mg Zn/kg down to 0.001 gamma Zn/kg and their effect on the blood sugar was examined: Zinc chloride, sulfate, acetate, pyrophosphate, citrate, tartrate, malate, maleinate, pyruvate, gluconate, glucuronate and ascorbinate. Similarly there were examined the complex zinc salts of glycocoll, alanine, glutaminic acid and some additional alpha-amino acids in doses of several mg Zn/kg down to 0.0001 gamma Zn/kg (including oral and intramuscular application besides intravenous).

Most of the tested zinc salts of N-free acids in the dosage range referred to can cause slight initial blood sugar increases, which however, do not occur regularly and whose degree does not depend on the dose. Even very small doses, such as 1 gamma and 0.01 gamma Zn/kg, can have a blood sugar increasinf effect.

Zinc-amino acid complexes in doses over 1 mg Zn/kg produce considerable hyperglycemia, possibly glucosuria, and are in most cases highly toxic. At doses under 1 mg Zn/kg differences appear in the structure of the complexes: From one and the same amino acid active and inactive zinc complexes can be prepared, which differ in their composition.

With highly active zinc complexes distinct blood sugar increases occurring shortly after the injection are still obtained with fractions of one gamma Zn/kg.

(Translated by Carl Demrick Associates,Inc/LH/t)

für eine teilweise Dephosphorylierung von Phosphopepton und x-Casein durch Phosphomonoesterase.

Magenkathepsin besitzt keine Phosphataseaktivität bei den untersuchten Substraten. Die enzymatische Dephosphorylierung von Phosphopepton und Casein wird durch gleichzeitigen kathep der Substrate kaum beeinflußt.

Blutzuckerwirkung von Zinkverbindungen

Von

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Über den Einfluß von Zinkverbindungen auf den Kohlenhydratstoffwechsel liegen, wenn man von zinkhaltigen Insulinpräparaten absieht, nur wenige Untersuchungen vor. 1892 berichteten italienische Autoren[1] über beträchtliche Glykosurie bei Hunden, die täglich mit dem Futter 0,5—1 g Zink erhielten. 1918 teilten Salant und Wise[2] mit, daß Fütterung oder Injektion von Zinksalzen bei Kaninchen, Hunden und Katzen Hyperglykämie und Glucosurie hervorrief. Bei oraler Zufuhr von Zinkacetat zeigten Kaninchen Glucosurie und Albuminurie bei 335 mg Zink/kg, aber noch nicht bei 30—100 mg Zn/kg. Subcutan verabfolgtes Zinkmalat rief in Dosen von 50—100 mg Zink/kg Glucosurie und Albuminurie hervor, intravenös ließ sich mit Dosen von 9—10 mg Zn/kg als Zinkmalat eben noch geringe Glucosurie erzeugen, wobei die Blutzuckerwerte meist um 200 mg % lagen. Fast alle Tiere wiesen Albuminurie auf und starben im Verlaufe von 2—9 Tagen nach der Zinkzufuhr. Katzen reagierten nach subcutaner Injektion von 25—100 mg Zn/kg als Zinkmalat mit Glucosurie; Hunde erhielten 15—26 mg Zn/kg als Zinkmalat intramuskulär, wobei meist Glucosurie auftrat. Doch überlebten diese Hunde die Zinkinjektion nur um 1—5 Tage.

Der Einfluß geringerer Dosen injizierter Zinksalze auf den Blutzucker von Hunden wurde von Sanfilippo[3] untersucht (je 0,87 mg Zn/kg i.m. und i.v. als Zink-chlorid, -bromid, -jodid, -nitrat, -sulfat, -lactat und -acetat). Er fand keine Veränderungen des normalen Glucosespiegels. Auch Berenshtein und Shkolnik[4] beobachteten nach subcutaner Injektion von Zink-sulfat oder -acetat in Dosen von 100—200 γ Zn/kg bei Kaninchen und Hunden keine Veränderungen des Blutzuckers, wohl aber nach höheren Dosen (0,5—5,0 mg Zn/kg).

Zusammenfassend ergibt sich aus diesen Untersuchungen, daß zur Erzeugung von Hyperglykämie und Glucosurie hohe Zinkdosen erforderlich sind. Diese sind jedoch zumeist stark toxisch, so daß die Tiere oft noch im akuten Versuch verenden. Bei Verabreichung geringerer

[1] L. d'Amore, C. Falcone u. L. Maramaldi, C. R. Séances Soc. biol. Filiales Associées 4, 335 [1892].
[2] W. Salant u. L. Wise, J. biol. Chemistry 34, 447 [1918].
[3] G. Sanfilippo, Arch. Farmacol. sperim. 73, 87 [1942].
[4] F. Y. Berenshtein u. M. J. Shkolnik, Fizio. Z. 37, 120 [1950]; zit. n.: Excerpta Med. 6, Sect. III, Nr. 10, 441 [1952]; Chem. Abstr. 45, 10330 [1951].

Zinkdosen verschwindet die Glucosurie, während die Hyperglykämie zunächst noch vorhanden ist, aber bei weiter herabgesetzter Dosis ebenfalls ausbleibt. Bei parenteraler Zufuhr dürfte nach den Angaben der Literatur diejenige Zinkdosis, welche noch deutliche Hyperglykämie auslöst, im Bereiche von etwa 1—5 mg Zn/kg liegen. Aus den genannten Untersuchungen geht aber nicht hervor, ob das Ausmaß des Zinkeffektes von der chemischen Struktur der zugeführten Zinkverbindung variiert wird oder ob deren Zusammensetzung für die hyperglykämisierende Wirkung ohne Bedeutung ist.

Wir untersuchten den Einfluß verschiedener, mit Zink verbundener Säurereste auf Höhe und Dauer der Zink-Hyperglykämie; insbesondere prüften wir, ob man mit geeigneten organischen Resten, z. B. mit komplexen Zinkverbindungen, eine Blutzuckersteigerung durch geringere Zinkdosen erreichen kann, d. h. ob auch Dosen von 1 mg Zn/kg bis herab zu Bruchteilen eines γ Zn/kg den Blutzucker noch beeinflussen können.

Die vorliegende Arbeit befaßt sich

1. mit Zinksalzen stickstofffreier Säuren, welche parenteral an Kaninchen in Dosen von 1 mg bis herab zu 0,001 γ Zn/kg verabreicht wurden. Folgende Zinksalze wurden geprüft:

Chlorid	Pyrophosphat	Malat	Gluconat
Sulfat	Citrat	Maleinat	Glucuronat
Acetat	Tartrat	Pyruvat	Ascorbinat

2. mit Zinksalzen von α-Aminosäuren, bei denen die Festigkeit der koordinativen Zinkbindung je nach Art und Zahl der Aminosäurereste weitgehend abgewandelt werden kann. In der Literatur waren keine Angaben über die Beeinflussung des Blutzuckers durch Zink-Aminosäure-Komplexe zu finden; wir prüften die orale, intramuskuläre und intravenöse Zufuhr von Zinkglykokoll, Zink-alanin und Zink-glutaminat, wobei Dosen von mehreren mg Zink/kg bis herab zu 0,001 γ Zn/kg herangezogen wurden.

Methodik und Substanzen

Alle Versuche an Kaninchen, die 24 Stdn. gehungert hatten. Lösen der zu injizierenden Zinkverbindungen in 0.9-proz. NaCl-Lösung, Einstellen des p_H auf 6,4—7,0. Verwendung nur frisch bereiteter Lösungen. Blutzuckerbestimmungen nach Hagedorn-Jensen oder nach Fujita und Iwatake[5]. Anzahl und zeitliche Abstände der Blutentnahmen sind aus den Abbildungen zu ersehen.

Die zu prüfenden Zinkverbindungen wurden mit Ausnahme von Zinkchlorid, -sulfat und -acetat (käufliche p.a.-Präparate) und Zinkpyrophosphat (s. u.) in fester Form dargestellt. Die Bestimmung des Zinkgehaltes erfolgte durch direkte Mikrotitration mit Äthylen-diamintetraessigsäure als Dinatriumsalz (Komplexon III) nach Flaschka[6]. Indikator: Eriochromschwarz T. Berechnung der für den Tierversuch erforderlichen Substanzmenge auf Grund der Zinkanalyse*.

[5] A. Fujita u. D. Iwatake, Biochem. Z. 242, 43 [1931].
[6] H. Flaschka, Mikrochemie 39, 38 [1952].
* Für die Ausführung der Zinkanalysen danken wir Fräulein Dr. A.-M. Fretzdorff (Medizinische Forschungsanstalt, Biochemische Abteilung).

Die Gewinnung der Zinksalze erfolgte durch Umsetzung der betreffenden in Wasser gelösten Säure mit der berechneten Menge Zinkcarbonat, gegebenenfalls mit Ausfällung des gebildeten Zinksalzes durch Äthanol. An Stelle des sehr schwerlöslichen, für Injektionszwecke ungeeigneten $Zn_2P_2O_7$ benutzten wir **saures Zinkpyrophosphat**, welches aus $ZnCO_3$ und wäßriger Pyrophosphorsäure gelöst erhalten wurde. In gleicher Weise wurden **Zinkcitrat** und **Zinkmalat** hergestellt und aus der wäßrigen Lösung durch Zusatz des gleichen Volumens Äthanol ausgefällt. **Zinktartrat, Zinkmaleinat** und **Zinkpyruvat** fielen spontan als amorphe Niederschläge aus Wasser aus; diese drei Salze konnten auf Grund ihrer Schwerlöslichkeit nur in Dosen von höchstens 100 γ bzw. 10 γ Zn/kg (s. u.) angewandt werden. **Zinkgluconat**, gewonnen aus Gluconsäure-lacton und $ZnCO_3$, fiel als körnig kristalliner Niederschlag an und entsprach der in der Literatur[7] angegebenen Zusammensetzung: $Zn(gluconat)_2 + 5\,H_2O$. Bei der Darstellung von **Zink-glucuronat** aus Glucuronsäurelacton und $ZnCO_3$ beachteten wir folgenden Umstand: um ein bei neutralem p_H nicht sogleich durch Hydrolyse zerfallendes Zinksalz zu gewinnen, wurde mit überschüssiger Glucuronsäure (Zn : Glucuronsäure = 1 : 3,5) angesetzt, wobei wir das Salz aus 50-proz. Äthanol in gelben Kristallen erhielten. **Zinkascorbinat** stellte uns in dankenswerter Weise die Fa. Hoffmann-La Roche, Basel, in Form eines gelben amorphen Pulvers mit einem Zinkgehalt von 18,3% zur Verfügung.

Glykokoll, Alanin und Glutaminsäure liefern je nach dem Herstellungsverfahren Zinksalze verschiedener Zusammensetzung, welche sich in Wasser bei neutraler Reaktion klar lösen oder auch durch Wasser mehr oder weniger rasch zerlegt werden unter Bildung flockiger Niederschläge. Zink-Aminosäure-Komplexe, die in wäßriger Lösung bei neutralem p_H sehr bald nach dem Auflösen sich trüben und Zinkhydroxyd entbinden, sind für Injektionszwecke ungeeignet. In unseren Versuchen benutzten wir nur solche Zink-Aminosäure-Komplexe, deren Stabilität gegenüber Wasser genügend groß war, um bei neutralem oder schwach alkalischem p_H einen Zerfall auszuschließen. Unter diesen bestehen jedoch hohe Wirkungsunterschiede; nur wenige Zink-Komplexe sind dazu geeignet, in Dosen unter 100 γ Zn/kg starke Blutzuckersteigerungen hervorzurufen. Die im folgenden besprochenen Versuchsergebnisse bei Zink-Aminosäuren beziehen sich, wenn nichts anderes vermerkt ist, auf **hochwirksame Komplexe.**

Ergebnisse

1. Zinksalze N-freier Säuren

Zinkchlorid und Zinksulfat

Die intravenöse Injektion von $ZnCl_2$ in Dosen von 1 mg, 100 γ, 10 γ, 1 γ und 0,01 γ Zink/kg führte nicht zu nennenswerten Blutzuckersteigerungen; nur einmal trat nach 1 mg Zink/kg im Anschluß an die Injektion ein Blutzuckeranstieg von 112 auf 132 mg% auf. Zwei andere Tiere wiesen nach der gleichen Dosis diesen Effekt jedoch nicht auf. Ähnlich verhielt sich $ZnSO_4$: 1 mg Zink/kg rief bei einem Tier direkt im Anschluß an die Injektion deutliche Hyperglykämie hervor mit einem Gipfel von 164 mg% nach 25 Min., ein zweites Tier reagierte auf die gleiche Dosis mit normalen Werten. Geringere Zinkdosen bis herab zu 0,01 γ Zink/kg (wie bei $ZnCl_2$) ließen deutliche und reproduzierbare blutzuckersteigernde Effekte vermissen.

Übereinstimmend mit den Literaturangaben[2,3,4] befindet man sich bei $ZnCl_2$ und $ZnSO_4$ mit 1 mg Zink/kg offenbar an der unteren

[7] Crieshammer, Arch. Pharmaz. 215, 204 [1879].

Grenze der hyperglykämisierenden Dosis. Dosen unter 1 mg Zink/kg zeigen keine deutliche Wirkung mehr, allerdings kann man gelegentlich auch bei sehr kleinen Dosen (z. B. 1 γ Zn/kg) noch geringe Blutzuckersteigerungen beobachten. Bemerkenswert ist, daß die Tiere die intravenöse Injektion dieser stark ionisierten Zinksalze ohne akute Erscheinungen vertragen; auch Spätschäden haben wir nicht feststellen können.

Zinkacetat

Im Vergleich zu $ZnCl_2$ und $ZnSO_4$ ändert sich das Bild der Blutzuckerkurven nach Zinkacetat i.v. insofern, als etwas häufiger, aber nicht sicher reproduzierbar, geringfügige Blutzuckersteigerungen auftreten (meist 20—30 mg%), die jedoch nicht dosisabhängig sind. Denn auch nach wenigen γ und sogar nach 0,001 γ Zn/kg als Zinkacetat i.v. lassen sich Blutzuckererhöhungen beobachten. Als Beispiel mögen die in Abb. 1 gegebenen Kurven für 0,01 γ und für 0,001 γ Zn/kg i.v. dienen.

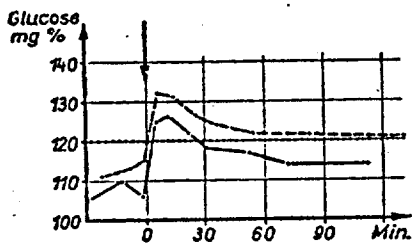

Abb. 1. 0,01 γ Zn als Zinkacetat i.v. —— K 288; 0,001 γ Zn als Zinkacetat i.v. ---- K 298.

Zinkpyrophosphat

Bei den Versuchen mit saurem Zinkpyrophosphat in Dosen von 1 mg Zn/kg bis 0,01 γ Zn/kg sahen wir in keinem Falle (es wurden 17 Tiere untersucht) Blutzuckersteigerungen, die über die normalen Schwankungen hinausgingen.

Zinkcitrat

Im Vergleich zu $ZnCl_2$, $ZnSO_4$ und Zinkacetat findet sich bei Zinkcitrat keine Zunahme des blutzuckersteigernden Effektes. Auf Grund von 14 Kaninchenversuchen haben wir vielmehr den Eindruck, daß Zinkcitrat nur in Ausnahmefällen den Blutzucker beeinflußt, dann aber durchaus nicht dosisabhängig wirkt, denn gerade die Dosen von 1 mg und 100 γ Zink kg erbrachten ausgesprochene Leerkurven.

Zinktartrat

Zinktartrat, welches aus Gründen der Löslichkeit nur in Dosen von 10 γ, 1 γ und 0,01 γ Zink/kg geprüft werden konnte, verhielt sich nicht anders als Zinkcitrat.

Zinkmalat

Die intravenöse Injektion von Zinkmalat (18 Kaninchenversuche) führte in etwa der Hälfte der Fälle bei allen geprüften Dosen zu Blutzuckersteigerungen. Als Beispiele geben wir in Abb. 2 Blutzuckerkurven, wie wir sie nach Injektion von 1 mg und 1 γ Zink/kg als Zinkmalat erhielten.

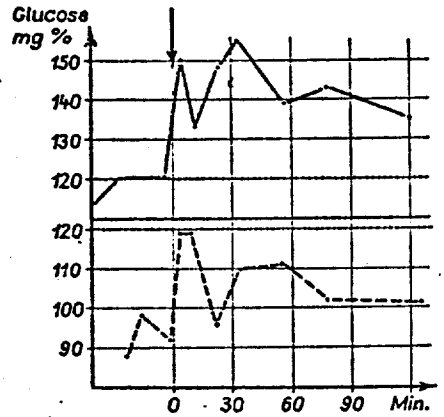

Abb. 2. 1 mg Zink/kg als Zinkmalat —— K 249; 1 γ Zink/kg als Zinkmalat - - - - K 248.

Zinkmaleinat

Bei Zinkmaleinat untersuchten wir wegen der Schwerlöslichkeit dieses Salzes die Dosis von 1 mg Zn/kg nicht, um die Injektion zu großer Volumina zu vermeiden. Die Dosen von 100 γ bis zu 0,01 γ Zn/kg führten in der Mehrzahl der Fälle zu initialen, aber so geringen Blutzuckersteigerungen, daß wir diese nicht mit Sicherheit auf die injizierten Zink-Komplexe zurückführen möchten.

Zinkpyruvat

Ebenso wie bei Zinkmaleinat ließ sich auch bei Zinkpyruvat wegen der Schwerlöslichkeit dieser Verbindung die Dosis von 1 mg Zn/kg nicht in ein für die Injektion geeignetes Volumen bringen. Die intravenöse Injektion von Zinkpyruvat in Dosen von 100 γ, 10 γ, 1 γ und 0,01 γ Zn/kg rief bei 12 Kaninchen keine verwertbare Beeinflussung des Blutzuckers hervor.

Zinkgluconat

Nach Injektion von Zinkgluconat (14 Kaninchen) traten deutliche Blutzuckersteigerungen nur selten auf. Bemerkenswert ist, daß gerade die niedrigsten Dosen (0,01 γ Zn/kg) die am besten ausgeprägten Blutzuckersteigerungen hervorriefen, siehe hierzu Abb. 3.

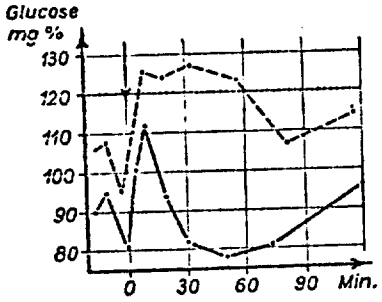

Abb. 3. Je 0.01 γ Zn/kg i.v. als Zinkgluconat. K 278 —— und K 315 - - - -.

Zinkglucuronat

Zinkglucuronat (14 Kaninchenversuche) verhält sich etwa ebenso wie Zinkgluconat. Als Beispiele geben wir in Abb. 4 zwei Kurven, welche den Blutzuckerverlauf nach Injektion von je 1 γ Zn/kg als Zinkglucuronat wiedergeben.

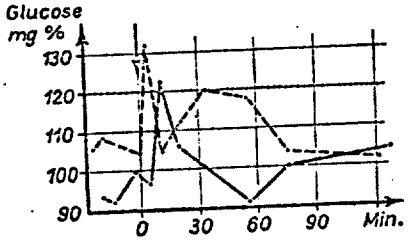

Abb. 4. Je 1 γ Zn/kg i.v. als Zinkglucuronat. K 242 —— und K 272 - - - -.

Zinkascorbinat

Das gleiche Ergebnis wie bei Zinkgluconat und -glucuronat brachten 24 Kaninchenversuche mit Zinkascorbinat. Man erhielt zum Teil fragliche, zum Teil aber auch eindeutige Blutzuckererhöhungen, die jedoch unabhängig von der Dosis auftraten. Als Beispiele geben wir in Abb. 5 den Blutzuckerverlauf nach Injektion von 100 γ Zn/kg und 0,01 γ Zn/kg als Zinkascorbinat.

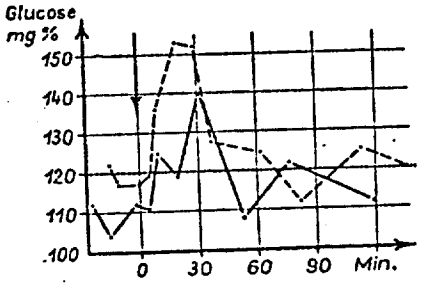

Abb. 5. 100 γ Zn/kg i.v. als Zinkascorbinat, K 324 ——; 0,01 γ Zn/kg i.v. als Zinkascorbinat, K 241 - - - -.

Kontrollversuche

Bei denjenigen der obengenannten Zink-Komplexe, welche sich als blutzuckerwirksam erwiesen, wurden Kontrollversuche mit den

entsprechenden freien Säuren oder deren Salzen ausgeführt. Natrium-malat, -glucuronat und -ascorbinat sowie freie Ascorbinsäure steigerten in den in Frage kommenden Dosen den Blutzucker nicht. Nur bei Natrium-gluconat kamen im Bereiche von 8 mg bis herab zu 8 γ Gluconsäure/kg geringe initiale Blutzuckersteigerungen vor, die jedoch bei entsprechenden Dosen von Calcium-gluconat nicht vorhanden waren.

2. Zinksalze von α-Aminosäuren

Die Versuchsresultate sind im folgenden nach der Höhe der pro kg Körpergewicht zugeführten Zinkmenge in drei Gruppen zusammengefaßt:

A. Mehr als 1 mg Zn/kg, B. 1 mg bis 1 γ Zn/kg, C. Weniger als 1 γ Zn/kg.

A. Dosen über 1 mg Zn/kg

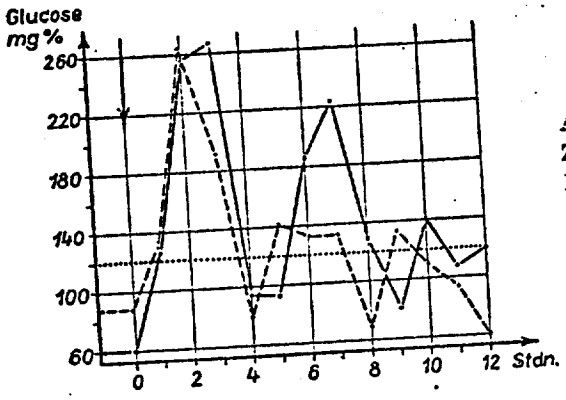

Abb. 6. 14 mg Zn/kg oral als Zink-glykokoll, K 60 ——; 10 mg Zn/kg oral als Zink-glutaminat, K 66 -·-·-.

Abb. 6 zeigt den Blutzuckerverlauf nach oraler Gabe von Zink-glykokoll und Zink-glutaminat (14 bzw. 10 mg Zn/kg): In den ersten 10 Stdn. nach Aufnahme der Zinkverbindung tritt erhebliche Hyperglykämie auf, begleitet von Glucosurie und Albuminurie. Auch in den folgenden Tagen wurden immer wieder hyperglykämische Zustände, unterbrochen von normalen und auch subnormalen Glykämie-Werten, beobachtet. Geringe Glucosurie war noch am 4. Tage nach der Zinkfütterung nachweisbar. Weitere Versuche mit Zink-glykokoll und Zink-glutaminat zeigten das gleiche Bild wie die in Abb. 1 dargestellten Kurven, deren Verlauf typisch für die Dysregulation des Blutzuckers bei Zinkvergiftung ist; denn die angewandten Dosen sind stark toxisch, fast alle damit behandelten Tiere gingen nach 2—10 Tagen zugrunde.

Salant und Wise[2] benötigten 335 mg Zn kg oral in Form des Zinkacetats, um beim Kaninchen Glucosurie zu erzeugen, mit 30—100 mg Zn/kg gelang dies nicht. Mit Zink-Aminosäure-Komplexen kann man, wie aus Abb. 6 hervorgeht, Glucosurie mit wesentlich geringeren Zinkdosen hervorrufen. Dies beruht sicherlich zum großen Teil darauf, daß im Gegensatz zum Zinkacetat die benutzten Zink-Aminosäure-

Komplexe sehr gut resorbiert werden. Aus stabilen Zink-glykokoll-Komplexen z. B. werden im Magen-Darm-Kanal kaum Zinkionen freigesetzt, die typischen Schwermetalleffekte fehlen. Ein bei neutralem p_H klar lösliches Zink-glykokoll z. B. ruft beim Menschen nach oraler Aufnahme weder Metallgeschmack noch Nausea oder Erbrechen hervor.

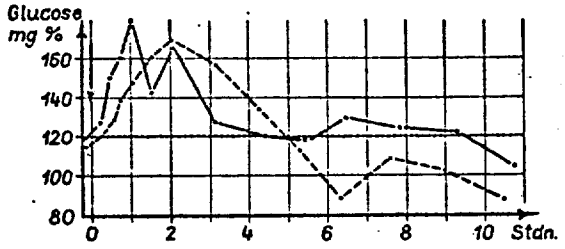

Abb. 7. Je 2,5 mg Zn/kg intramuskulär als Zink-alanin, K 207 —— und K 295 - - - -.

In Abb. 7 sind zwei Blutzuckerkurven nach intramuskulärer Zufuhr von je 2,5 mg Zn/kg als Zink-alanin dargestellt. 15 Min. nach der Injektion ist die beginnende Blutzuckersteigerung bereits nachweisbar; die Hyperglykämie erstreckt sich über 4—5 Stunden. Der Verlauf der Blutzuckerkurve führt 6—9 Stdn. nach der Injektion bei dem einen Tier (K 207) nochmals zu leicht hyperglykämischen Werten. Diese Tiere blieben am Leben.

Die intramuskuläre Zufuhr von Aminosäure-Zink-Komplexen in höheren Dosen, z. B. 28 mg Zn/kg als Zink-glykokoll oder 20 mg Zn/kg als Zink-glutaminat, wirkte sich auf Blutzucker und Allgemeinzustand der Tiere gleichartig aus wie oben unter Abb. 6 für die oralen Gaben beschrieben; von 6 Tieren gingen 5 nach 3—5 Tagen zugrunde.

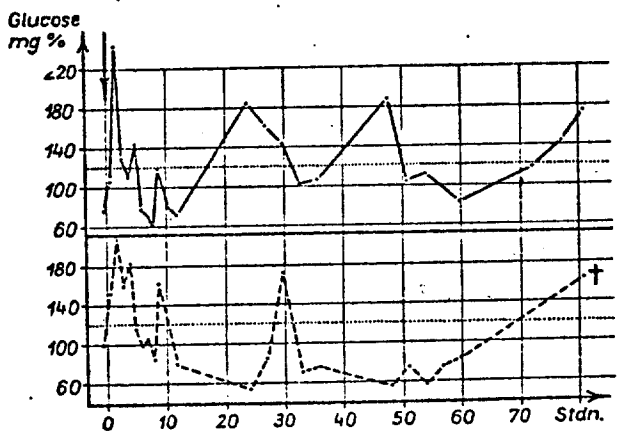

Abb. 8. 6 mg Zn/kg intravenös als Zink-glutaminat, K 65 ——; 8,5 mg Zn/kg intravenös als Zink-glykokoll, K 62 - - - -.

Abb. 8 zeigt den Verlauf der Blutzuckerkurven bei intravenöser Injektion von mehreren mg Zn als Zink-glykokoll (8,5 mg Zn/kg) und Zinkglutaminat (6 mg Zn/kg). In beiden Fällen beobachtet man hyperglykämische Werte, ebenso Glucosurie und Albuminurie, noch 80 Stdn. nach der Injektion, dazwischen treten Blutzuckersenkungen bis herab zu rd. 50 mg% auf. Die intravenöse Zufuhr von Zinkmengen über 5 mg Zn/kg in Form innerer Zink-Komplexe von α-Aminosäuren führte bei 7 von 8 Tieren zum Tode. K 62 in Abb. 8 ging nach 80 Stdn. verloren, in Parallelversuchen mit der gleichen Dosis gingen die Tiere 60 bzw. 100 Stdn. nach der Injektion zugrunde. K 65 (s. Abb. 8; 6 mg Zn/kg) überstand als einziges die Zinkbelastung und blieb am Leben.

Bei intravenöser Gabe von 5 bis 1 mg Zn/kg wurden Ausmaß und Dauer der Hyperglykämie geringer, zugleich ließ die Toxizität bedeutend nach, von 5 Tieren blieben 4 am Leben.

B. Dosen von 1 mg bis 1 γ Zink/kg

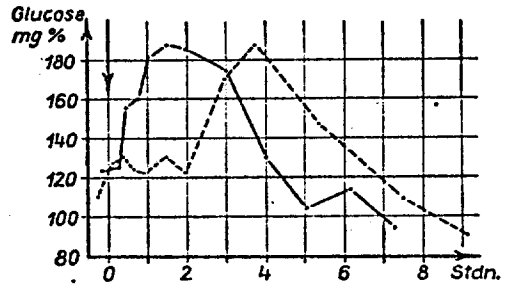

Abb. 9. 1 mg Zn/kg oral als Zink-glykokoll, K 293 ——; 50 γ Zn/kg intramuskulär als Zink-glykokoll, K 288 - - - -.

Wie aus Abb. 9 hervorgeht, ruft bei intramuskulärer Zufuhr noch die Dosis von 50 γ Zn/kg als Zink-glykokoll ausgeprägte, mehrere Stunden anhaltende Hyperglykämie hervor. Auf oralem Wege kommt 1 mg Zn/kg als Zink-glykokoll ebenfalls deutlich zur Wirkung. Die geringste noch wirksame Dosis bei oraler Zufuhr wurde wegen der unübersichtlichen Resorptionsverhältnisse des Kaninchendarms nicht getestet.

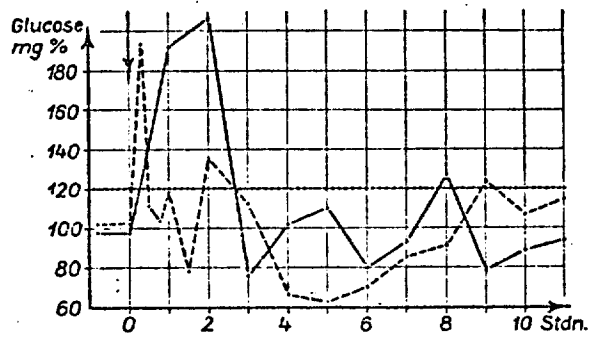

Abb. 10. 850 γ Zn/kg intravenös als Zink-glykokoll, K 76 ——; 141 γ Zn/kg intravenös als Zink-glykokoll, K 90 - - - -.

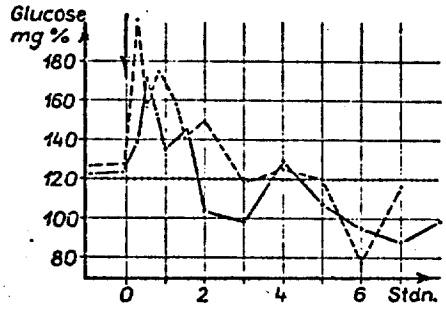

Abb. 11. 14,1 γ Zn/kg intravenös als Zink-glykokoll, K 78 - - - -; 3,5 γ Zn/kg intravenös als Zink-glykokoll, K 96 ——

Die Abbildungen 10 und 11 zeigen Blutzuckerkurven nach intravenöser Injektion von Zink-glykokoll im Bereiche von 850 γ bis 3,5 γ Zn/kg. Auch diese Zinkdosen rufen noch erhebliche Hyperglykämie hervor, die bereits 15 Min. nach der Injektion nachweisbar ist und innerhalb der ersten Stunde Werte zwischen 160 und 200 mg% erreichen kann. Im allgemeinen halten die Blutzuckersteigerungen auch jetzt noch mehrere Stunden nach der Injektion an.

C. Geringere Dosen als 1 γ Zink/kg

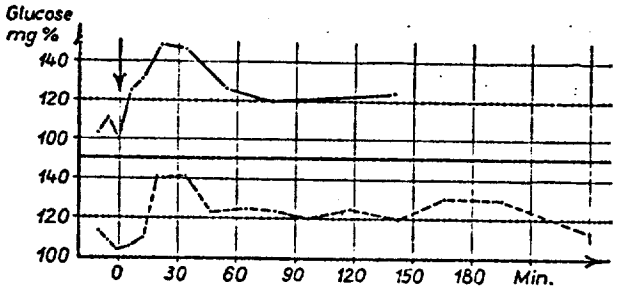

Abb. 12. 0,5 γ Zink/kg intramuskulär als Zink-glykokoll, K 246 - - - -; 0,5 γ Zink/kg intravenös als Zink-glykokoll, K 296 ——.

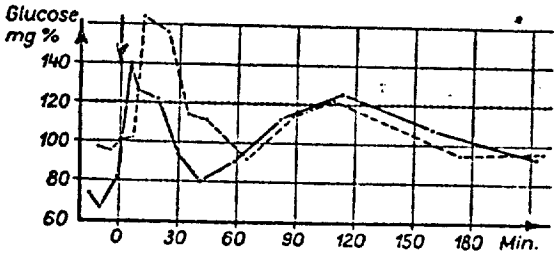

Abb. 13. 0,01 γ Zn/kg intravenös als Zink-glykokoll, K 276 - - - -; 0,001 γ Zn/kg intravenös als Zink-glykokoll, K 203 - - - -.

In den Abbildungen 12 und 13 sind Versuche mit parenteraler Zufuhr von weniger als 1 γ Zn/kg dargestellt. Mit der Herabsetzung der Zinkdosis auf immer kleinere Mengen gehen Höhe und Dauer der Blutzuckersteigerung zurück, die Hyperglykämie manifestiert sich als steiler, kurz dauernder Blutzuckeranstieg, der fast immer sofort nach der Injektion auftritt und dem häufig noch eine zweite, geringere Nachschwankung folgt.

Die initiale Hyperglykämie nach diesen erstaunlich geringen Zinkdosen tritt je nach Art des verabreichten Zink-glycin-Komplexes mehr oder weniger regelmäßig, bei den am besten geeigneten Zink-glycin-Komplexen nahezu ausnahmslos auf (70 Kaninchenversuche mit Dosen unter 1 γ Zn/kg als Zink-glykokoll). Die unterste Wirkungsgrenze wurde nicht ausgetestet, ein Stichversuch mit 0,0001 γ Zn/kg des bestwirksamen Zinkglycin-Komplexes rief noch deutlichen Blutzuckeranstieg hervor.

Besprechung der Ergebnisse

1. Zinksalze N-freier Säuren

Im Vergleich zu den eingangs erwähnten Literaturangaben[1, 2, 4] über Hyperglykämie und Glucosurie nach Zufuhr hoher und toxischer Zinkmengen zeigen die vorliegenden Untersuchungen, daß Zinksalze stickstofffreier Säuren in Dosen von 1 mg Zink/kg i.v. und darunter geringe Blutzuckersteigerungen auslösen können, die jedoch nicht regelmäßig auftreten. Dabei ist bemerkenswert, daß kleinste Dosen, z. B. 1 γ oder 0,01 γ Zn/kg, noch initialen Anstieg des Blutzuckers hervorrufen können und daß im Bereich von 1 mg bis 0,01 γ Zn/kg i.v. keine Dosisabhängigkeit besteht. Auf Grund von Kontrollversuchen mit den freien Säuren bzw. ihren Natriumsalzen muß der blutzuckererhöhende Effekt dem Zink zugeschrieben werden.

Die Wirkungsunterschiede zwischen anorganischen und organischen Zinksalzen oder zwischen stark komplexen, schwach komplexen und nicht komplexen Zinksalzen sind nicht genügend stark ausgeprägt, um sichere Rückschlüsse zuzulassen auf etwaige Zusammenhänge zwischen Art der Metallbindung und blutzuckersteigernder Wirkung. Trotzdem vermitteln die Kurven den Eindruck, daß für die Wirkung des Zinks auf den Blutzucker die Struktur des mit dem Metall verbundenen Säurerestes nicht gleichgültig ist. Besonders auffallend und biologisch bemerkenswert erscheint uns die Tatsache, daß sehr kleine Zinkdosen, z. B. 1 γ oder 0,01 γ Zink/kg, den Blutzuckerspiegel beeinflussen können, denn es handelt sich dabei um Zinkmengen, die im Bereich physiologischer Größenordnungen liegen und die in unseren Versuchen fast durchweg an zellvertraute Säurereste gebunden sind.

Die von uns verabreichten Zinkdosen, welche 1 mg Zn/kg nicht überschritten, ließen keinerlei Toxizität erkennen. Beim Hund beob-

achteten jedoch Vallee und Mitarb.[8] nach intravenöser Injektion von 4 mg/kg Zinkgluconat Lähmungen der Hinterläufe, herabgesetzte Sehnenreflexe und allgemeine Schlaffheit, während 2 mg/kg Zinkgluconat sowohl vom Hund als auch vom Menschen gut vertragen wurden.

2. Zinksalze von α-Aminosäuren

Ein Überblick über die mit Zink-Aminosäure-Komplexen erhobenen Befunde ergibt ein wesentlich anderes Bild im Vergleich zu den nur schwach ausgeprägten blutzuckersteigernden Eigenschaften der Zinksalze stickstofffreier Säuren.

Betrachtet man zunächst diejenigen unserer Versuche mit Zink-Aminosäure-Komplexen, in denen mehr als 1 mg Zink/kg verabreicht wurde, so findet man in allen Fällen beträchtliche Hyperglykämie, zum Teil auch Glucosurie. Dabei sind im Vergleich zu den von Salant und Wise[2] angewandten Zinkdosen — 335 mg Zn/kg als Acetat oral, 25—100 mg Zn/kg als Malat subcutan, 15—26 mg Zn/kg als Malat intramuskulär und 9—10 mg Zn/kg als Malat intravenös — die in unseren Versuchen zugeführten Zinkmengen wesentlich geringer; die unterste Zinkdosisgrenze der amerikanischen Autoren entspricht ungefähr der oberen Grenze der von uns geprüften Zinkdosen. Die gegenüber den Zinksalzen stickstofffreier Säuren beträchtlich höhere Wirkung der Zink-Aminosäure-Komplexe erklärt sich bei oraler Zufuhr, wie bereits erwähnt, sicherlich zum Teil aus der guten Resorbierbarkeit dieser Verbindungen.

Die Maskierung des Schwermetalls durch feste koordinative Bindung, wie sie in den benutzten Zink-Aminosäure-Komplexen vorliegt, trägt aber offensichtlich auch bei parenteraler Zufuhr zur Erhöhung der Zink-Wirkung bei. Eine Variation der Zinkwirkung durch die Art des Aminosäure-Restes haben wir bei Dosen über 1 mg Zn/kg nicht beobachten können, soweit es sich um feste Komplexe handelte, die bei neutralem p_H klar gelöst blieben. Zink-glykokoll, Zink-alanin, Zink-glutaminat unterschieden sich in mg-Dosen in ihrer hyperglykämisierenden Wirkung praktisch nicht. Glucosurie war stets nur dann zu beobachten, wenn zugleich Hyperglykämie vorlag; eine Herabsetzung der Nierenschwelle für Glucose ließ sich nicht nachweisen.

Während Zink-Aminosäure-Komplexe in Dosen zwischen 1 und 10 mg Zn/kg oft über mehrere Tage anhaltende Dysregulation des Blutzuckers hervorrufen, ändern sich die Versuchsergebnisse bei Anwendung von Zinkdosen unter 1 mg Zn/kg bis herab zu Bruchteilen eines γ Zn/kg in dreifacher Richtung:

1. Höhe und Dauer der Hyperglykämie nehmen mit Herabsetzung der Zinkdosis ab, doch tritt der Blutzuckeranstieg weiterhin im An-

[8] B. L. Vallee, R. G. Fluharty u. J. G. Gibson, IV. Internat. Cancer Research Congress, zit. n. Physiol. Rev. 29, 375/376 [1949].

schluß an die Injektion auf. Bei Dosen unter 1 γ Zn/kg ist die Dosisabhängigkeit nicht mehr feststellbar.

2. Mit fortlaufender Erniedrigung der Dosis nimmt die Toxizität immer mehr ab.

3. Feinheiten im komplexchemischen Bau machen sich zusammen mit der Spezifität des Aminosäurerestes bemerkbar.

Davon verdient 3. besondere Beachtung, da sich hier Hinweise auf die Zusammenhänge zwischen spezieller Komplex-Struktur und hyperglykämisierender Wirkung ergeben. Für die Auslösung eines Blutzuckeranstiegs durch Zink-Aminosäure-Verbindungen in γ-Dosen ist, wie bereits erwähnt, Grundbedingung, daß es sich um Komplexe handelt, die bei neutralem p_H in wäßriger Lösung auch bei mehrtägigem Stehenlassen kein Zinkhydroxyd entbinden. Andererseits ergab die Prüfung einiger Zink-Aminosäure-Komplexe mit besonders fester Metallbindung, wie z. B. Zink-asparagin, Zink-histidin, Zink-histidyl-histidin, Zink-cystein und Zink-glutathion, daß diese Verbindungen in Dosen unter 1 mg Zn/kg den Blutzucker nicht beeinflussen. Aber auch vom Glykokoll lassen sich stabile Zink-Komplexe darstellen, die in Gaben von einigen 100 γ Zn/kg nicht blutzuckersteigernd wirken.

Vergleicht man die Zink-Aminosäure-Komplexe mit den Zinksalzen N-freier Säuren (s. o.) hinsichtlich des hyperglykämisierenden Effektes, so ergibt sich folgendes Bild: Während die Blutzuckersteigerungen nach Verabreichung von Zinksalzen N-freier Säuren in Dosen von 1 mg Zn/kg und darunter sehr gering sind, beobachtet man bei den gleichen Dosen geeigneter Zink-Aminosäure-Komplexe wesentlich stärkere und regelmäßig auftretende Effekte; zudem besteht hier im Bereich von 10 mg Zn/kg bis herab zu etwa 1 γ Zn/kg Abhängigkeit zwischen Höhe der Dosis und Ausmaß der Wirkung. Bei Dosen von 1 γ bis 0,001 γ Zn/kg läßt sich eine Abstufung der Wirkung je nach der zugeführten Zinkmenge nicht mehr erkennen; offenbar wird der Effekt dann durch individuelle Unterschiede in der jeweiligen Stoffwechsellage der Versuchstiere stärker beeinflußt.

Kontrollversuche mit Aminosäuren: Im Schrifttum[9, 10, 11] finden sich Beobachtungen über hyperglykämisierende Wirkungen von Aminosäuren, wobei allerdings zur Erzeugung von Blutzuckersteigerungen unvergleichlich höhere Gaben erforderlich sind als dies in unseren Versuchen der Fall ist. Die von uns ausgeführten Kontrollversuche mit Aminosäuren (Prüfung aller Aminosäuren, die auch als Zinksalze verabreicht wurden, in entsprechender Dosierung) zeigten, daß die mit Zink-Aminosäure-Komplexen erreichten Blutzuckersteigerungen auf das komplex gebundene Zink zurückzuführen sind.

[9] L. Pollak, Biochem. Z. 127, 120 [1922].
[10] M. Chikano, Biochem. Z. 205, 154 [1929].
[11] E. G. Schenck, Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 167, 201 [1932].

Ein Beweis hierfür ist auch die oben erwähnte Tatsache, daß wir von der gleichen Aminosäure Zink-Komplexe verschiedener Zusammensetzung in Händen haben, von denen bei Dosen unter 1 mg Zn/kg einige wirksam sind, während andere den Blutzucker nicht beeinflussen.

Toxizität: Während bei der Prüfung der Zinksalze N-freier Säuren die zugeführten Dosen (nicht höher als 1 mg Zn/kg) keine Schädigungen der Versuchstiere erkennen ließen (s. o.), riefen die in Mengen von mehr als 1 mg Zn/kg verabreichten Zinksalze von Aminosäuren in vielen Fällen ausgeprägte toxische Symptome hervor, die aber auch bei Dosen von 1 mg Zn/kg und darunter nicht ganz ausblieben. Unsere Beobachtungen zur Toxizität dieser Verbindungen seien kurz zusammengefaßt:

Das Allgemeinverhalten der Versuchstiere nach Injektion von Zink-Aminosäure-Komplexen ist abhängig von der Höhe der Zinkdosis. Zinkgaben über 1 mg Zn/kg als Zink-glykokoll oder Zink-glutaminat führten zu einem mit steigender Dosis immer schwerer werdenden und zum Teil mehrere Tage andauernden kollapsartigen Zustand, in dem aus den kalten Ohren nur unter großen Schwierigkeiten Blut zu gewinnen war. Die Tiere saßen geduckt, ihre Atmung war beschleunigt. Etwa zwei Drittel der Kaninchen kamen aus diesem Zustand nicht mehr heraus und gingen nach 48 bis 120 Stdn. zugrunde, nachdem bei den meisten von ihnen zuvor noch Paresen der Hinterläufe, teilweise auch Blasenlähmung, aufgetreten waren. Dosen von wenigen mg Zn/kg führten in mehreren Fällen erst nach 8—14 Tagen zum Tode der Tiere in stark reduziertem Ernährungszustand. Von den übrigen Kaninchen vertrug nur ein geringer Teil die genannten Zinkdosen ohne Beeinträchtigung ihres Allgemeinzustandes, der Rest erholte sich langsam und überlebte ohne erkennbare Spätsymptome. Für das Ausmaß der toxischen Erscheinungen war bei hohen Zinkdosen die Applikationsweise — i.v., i.m. oder per Magensonde — gleichgültig.

Zinkgaben von 1 mg bis etwa 20 γ/kg in Form der oben genannten Aminosäure-Komplexe riefen wesentlich geringere Beeinträchtigungen des Kreislaufs hervor, die parallel zur Zinkgabe im Ausmaß abnahmen. Mit Herabsetzung der Dosis stieg weiterhin die Zahl der Tiere, die keine toxischen Erscheinungen erkennen ließen. Wenige Tiere starben in reduziertem Zustand nach 10—14 Tagen.

Zinkdosen unter 20 γ/kg führten nur in Ausnahmefällen zu toxischen Allgemeinveränderungen. Als solche Ausnahme mag das Auftreten einer kompletten schlaffen Lähmung der Hinterläufe mit Blasenlähmung 15 Min. nach Injektion von 0,35 γ Zn/kg i.v. als Zink-glykokoll (K 122) angeführt sein.

Für die vergleichbaren Dosen von 1 mg Zn/kg und darunter ergibt sich daraus, daß Zinksalze von Aminosäuren im allgemeinen toxischer sind als Zinksalze stickstofffreier Säuren.

Über den Wirkungsmechanismus, welcher die Blutzuckersteigerung durch Zinkverbindungen hervorruft, lassen sich heute noch

keine sicheren Aussagen machen. Hinweise auf einen möglicherweise zugrundeliegenden Vorgang können vielleicht ältere Untersuchungen von Häusler und Schnetz[12] geben: Diese Autoren untersuchten an der isolierten Froschleber den Einfluß von Metallen auf die normale und auf die durch Adrenalin gesteigerte Glykogenolyse. Sie fanden, daß Zink (neben Cu und Hg) in bestimmten Konzentrationsbereichen (10^{-4} bis 10^{-6} Millimol Metallsalz/l in der Durchspülungsflüssigkeit) die Zuckerabgabe aus der Froschleber deutlich steigert, nicht aber in anderen, höheren (10^{-2} bis 10^{-3} Millimol $ZnSO_4/l$) oder geringeren (10^{-7} Millimol $ZnSO_4/l$) Konzentrationsbereichen. Wurde dagegen gleichzeitig mit dem Metall Adrenalin der Durchspülungsflüssigkeit zugesetzt, so hemmte Zink die im Leerversuch durch Adrenalin hervorgerufene Steigerung der Glykogenolyse vollständig. Aus diesen Versuchen von Häusler und Schnetz[12] ergibt sich, daß Zink in sehr geringen Konzentrationen zumindest an der isolierten Kaltblüterleber glykogenolytisch wirken kann, wobei dieser Effekt ohne Beteiligung von Adrenalin zustande kommt.

In weiteren Versuchen kam Schnetz[13] jedoch zu der Auffassung, daß Zink-, Cadmium- und Kupfersalze am Ganztier die Adrenalin-Hyperglykämie deutlich vermindern und daß der normale Blutzuckerspiegel „durch die genannten Metalle nicht wesentlich beeinflußt" wird. Dies steht im Gegensatz zu den Befunden von Berenshtein und Shkolnik[4], welche eine Steigerung der Adrenalin-Hyperglykämie beobachteten, wenn zugleich $ZnSO_4$ injiziert wurde.

Es liegt nahe, zur Deutung der von uns beobachteten Blutzuckersteigerungen nach Zufuhr sehr geringer Zinkdosen an eine Beeinflussung entsprechender Fermentsysteme zu denken, welche Zink im Molekül enthalten bzw. durch Zink aktiviert werden. Hier kommen z. B. Phosphatase-Aktivierungen in Betracht, da ganz allgemein die Phosphat-Übertragung durch zweiwertige Metalle gesteigert wird und Zink in der wirksamen Gruppe von Phosphatasen enthalten sein soll[14, 15, 16].

Angesichts der spärlichen und uneinheitlichen Angaben im Schrifttum lag uns daran, zur Frage des hyperglykämisierenden Effektes von Zinkverbindungen eine sichere experimentelle Grundlage zu schaffen und darüber hinaus die bisher nicht bekannten blutzuckersteigernden Effekte sehr geringer Zinkdosen zu untersuchen. Die in der vorliegenden Arbeit enthaltenen 13 Abb. gingen aus etwa 500 Kaninchenversuchen hervor und sind jeweils für eine größere Versuchsreihe charakteristisch.

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[12] H. Häusler u. H. Schnetz, Biochem. Z. 275, 204 [1935].
[13] H. Schnetz, Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 178, 420 [1935]; Klin. Wschr. 15, 646 [1936].
[14] R. Cloetens, Biochem. Z. 308, 37 [1948].
[15] L. Massart u. L. Vandendriessche, Naturwiss. 28, 143 [1940]; R. Dufait u. L. Massart, Naturwiss. 29, 651 [1941].
[16] V. Sadasivan, Arch. Biochemistry 28, 100 [1950]; Nature [London] 170, 421 [1952].

Wie oben gezeigt wurde, lassen sich mit den Zinksalzen von 12 N-freien Säuren in Dosen von 1 mg Zn/kg und darunter keine stark ausgeprägten Effekte auf den Blutzuckerspiegel erzielen; in Anbetracht unserer zahlreichen Versuche möchten wir dieses Urteil als abschließend betrachten. Dagegen verlangen die Zink-Aminosäure-Komplexe eine weitere intensive Bearbeitung, vor allem auch in chemischer Hinsicht, da bisher ihre Wirksamkeit in γ-Dosen ebensowenig bekannt war wie die Tatsache, daß Zink-Aminosäure-Komplexe der gleichen Aminosäure, aber verschiedener Zusammensetzung, sich biologisch different verhalten können. Der letztere Sachverhalt macht sich bei hohen Dosen (über 1 mg Zn/kg) noch nicht deutlich bemerkbar, während man bei geringeren Dosen (unter 1 mg Zn/kg) erhebliche Wirkungsunterschiede bei den einzelnen Komplexen beobachten kann. In einer weiteren Arbeit werden wir über die Ergebnisse berichten, die wir bei der Untersuchung der Zusammenhänge zwischen hyperglykämisierender Wirkung und Struktur der koordinativen Zinkbindung erhalten haben.

Die weitere Verfolgung dieser Zusammenhänge dürfte von besonderem Interesse für das Verständnis des biologischen Verhaltens zinkhaltiger Naturstoffe sein, z. B. von Hormonen und Fermenten; denn wir besitzen in den Zink-Aminosäure-Komplexen einfache Modellsubstanzen, bei denen die möglichen Variationen der komplexen Zinkbindung relativ gut übersehbar und präparativ leicht zugänglich sind. Auf Grund unserer Versuchsergebnisse hat man ebenso wie bei anderen Metallen nun auch beim Zink damit zu rechnen, daß Feinheiten der komplexen Struktur in sehr hohem Maße das Verhalten von Zinkverbindungen im Stoffwechsel beeinflussen können. Infolgedessen führen die in der vorliegenden Arbeit mitgeteilten Befunde über die blutzuckersteigernde Wirkung von γ-Dosen geeigneter Zink-Aminosäure-Komplexe zu der Frage, ob auch die Natur von diesem blutzuckersteigernden Prinzip Gebrauch macht. Dies scheint der Fall zu sein, denn wir[17] konnten inzwischen zeigen, daß hyperglykämisierende Extrakte aus Pankreas und Magenschleimhaut regelmäßig komplex gebundenes Zink enthalten, welches in diesen Extrakten offenbar an der blutzuckererhöhenden Wirkung beteiligt ist.

Für verständnisvolle und unermüdliche Mitarbeit danken wir Frau Gertrud Langenberg und Fräulein Käthe Büttner.

Zusammenfassung

Die folgenden Zinksalze wurden an Kaninchen in Dosen von 1 mg Zink/kg bis herab zu 0,001 γ Zink/kg intravenös verabreicht und ihr Einfluß auf den Blutzucker untersucht: Zink-chlorid, -sulfat, -acetat, -pyrophosphat, -citrat, -tartrat, -malat, -maleinat, -pyruvat, -gluconat, -glucuronat und -ascorbinat. In gleicher Weise wurden geprüft die komplexen Zinksalze von Glykokoll, Alanin, Glutaminsäure und einiger

[17] G. Weitzel und Mitarbb., Naturwiss., 40 [1953], im Druck.

Effect of alkali metal-ions on the basic metabolism

By Ladislaus Berta and Gustav Gyceri

(From the Rheumatic- and Balneological Research Institute of the Therapy Commission Budapest, affiliated with the General Pathological Institute of the University) (Received on November 3, 1941)

If we study the effects of mineral waters, the question arises what influence do these waters have on the basic metabolism. In order to recognize their total effect we have to study first their individual ions separately. In this paper we report about our investigations regarding the effect of magnesium and sodium.

Tests which were made at the Institute by Kliebert (7) proves that rabbits which were fed with magnesium- and sodium- containing mineral water react to an adrenalin injection with a hyperglycemia of lower grade and shorter duration than the untreated controls. At the same time the sugar load curve obtained with the administration of dextrose is much higher, which can be accounted for by a better storage of glycogen in the liver.

Francke's tests (2) also show storage of glycogen in the liver after administration of Mg-gluconate. According to Hazard and Vaille (9), a small amount of magnesium is sufficient to reduce the blood sugar. This is also proven by tests by Land and Rigo (10), Kliebert (7) explains the action of Mg observed in his tests as a change of the vegetative nervous system in parasympathic direction.

On the basis of these considerations it seems interesting to peruse the literature from the viewpoint of the influence of a parasympathic preponderance on the basic metabolism. According to Lubbe and Rubinstein (11), the parasympathicomimetic acetylcholine and the sympathicus-paralyzing yohimbin reduce equally the basic metabolism. The same authors saw a similar effect with ergotamine. According to Orestano (12) ergotamine reduces the basic

metabolism likewise and when administered together with adrenalin it prevents its increasing effect. All these authors agree that adrenalin increases the basic metabolism and they consider the effect of atropine and pilocarpine as a two-phase effect.

On the basis of the above mentioned data we must conclude that the basic metabolism is reduced by the administration of magnesium salt if the magnesium actually causes a displacement of the vegetative equilibrium of the organism in parasympathic direction. No similar studies are known to us with regard to the effect of Na. We administered Mg and Na in the form of their gluconates assuming that the gluconate as an anion behaves indifferently and that the effect of the cation can therefore manifest itself freely.

The tests were divided into two groups: in the first group we observed the change in the basic metabolism following immediately a single injection of Mg or Na in the second group, however,we observed the changes occurring after long-term feeding with Mg or Na-gluconate.

The basic metabolism was determined partly with the original metabolism apparatus for small animals by Belak-Illenyi (13), and partly with the modification indicated by Berta, which was described in detail in the preceding study.

Among the 15 normally developed male rats weighing 160 to 220 g which were used for the tests, four animals proved completely suitable for metabolism tests. On these animals we made three times a test lasting 2 hours to determine the normal basic metabolism after fasting for 16-20 hours. According to the statistical calculations, the deviations from the mean were very small - the errors were 1/100 or 1/200 of the mean value, so that the reliability of our mean value is beyond any doubt.

In order to avoid that the seasonal changes in the diet would influence the basic metabolism of our rats, they were kept on a constant diet which consisted of a barley and corn mixture to which casein and fat were added.

In order to cover the vitamin need, it contained butter and yeast, the necessary amount of salt was provided by the salt mixture by McCallum. The tests were always carried out on the animals after fasting for 16-20 hours.

1. Magnesium tests to test the acute effect

In the following test series we injected 1.5-2.0 cc 10% magnesium-gluconate solution subcutaneously and determined after the administration for 6 hours in half-hour intervals the basic metabolism of the animals. In the rats no.5, 10,13 we made three tests this way, in no. 7 only one test because the animal died.

After the injection of Mg gluconate, the basic metabolism of the rat is reduced, table 1. This reduction reaches its low point in the 3rd to 4th half hour in which the O2-consumption drops to 53-83%, assuming a normal O2 consumption of 100%, so that we can only observe a reduction of 17-47%. This reduction was observed in each test. Then the basic metabolism starts to rise again and attains in the 5th to 8th half hour the normal level, sometimes it even rises above it, to settle thereafter at the normal level after more or less great fluctuations. In another part of the cases it remains constant at the normal level.

The Mg-gluconate solution used in this test series can be considered in osmotic respect substantially as a physiological solution. Due to their general physical effect, these salt solutions leave the basic metabolism either unchanged or they increase it slightly. An observation to the effect that the basic metabolism is physically reduced can not be found in the literature at all so that our finding can only be due to the chemical action of Mg-gluconate. The gluconate, however, is likewise ineffective as an anion, as it was proven by our later tests, so that the reduction of the basic metabolism must be ascribed to the Mg-effect.

In the course of our tests the question arose whether the effect observed here was not perhaps a result of the narcotic, sleep-inducing, tranquilizing

effect of magnesium; but this is not likely, because the mobility of the rats did not change after the injection of magnesium according to our observations. We should mention that in the rabbit at least 3 g/kg $MgSO_4$ produce narcosis according to Matthei and Butturini (14). This amount corresponds to about 0.6 g, converted to pure magnesium, but we used only 0.05 g Mg. From this small dose we can thus not assume a narcotic effect. With regard to the effect of narcotics on the basic metabolism, we should mention Lee Mitton's tests (15), according to which 0.05 g/kg isoamylethyl barbituric acid- hence a very strong narcotic dose - causes a reduction of 5-18%. 0.60-0.90 g/kg urethane - likewise a very large dose- cause only a reduction of less than 10%. The reduction of the basic metabolism observed in our tests can therefore not be accounted for by the narcotic effect of Mg.

In a similar sense have to be evaluated the tests by Tangl and Verzar (16) who found that the basic metabolism can be reduced by curare only in those animals which were not used to the determination of the metabolism, but not in animals which were used to them. Our animals, which went through a great number of tests, must therefore be considered as used to these tests.

II Magnesium feeding tests

In the second group of our tests we tested the changes of the basic metabolism after long-term administration of Mg.

We selected 20 rats for our tests. In general the normal basic metabolism was determined 10 times each in 2 hour tests, and we selected for the main test those four animals where the probable error (p.e.) of the mean value (M) was less than 2%. The metabolism of these animals is sufficiently constant to permit observation of the deviation from the normal value. During the entire test series the animals were kept on McCallum's normal diet, which we dosed exactly so that the animals received daily 7.5% of their body weight. After determining the normal basic metabolism, the animals were fed with Mg and their basic metabolism was determined. To this end we made again ten

tests. After the completion of ten tests, the administration of Mg was stopped and we observed for 5 weeks the return of the basic metabolism to the normal value, making one determination a week.

We selected for our tests as test material a Mg gluconate-solution which contained 5 mg pure Mg. The animals received then 0.01 cc per g body weight mixed into their normal diet. This way 0.05 mg pure Mg were fed daily per g body weight.

The determinations were made in the identical manner as described above. The results of our tests are compiled in table II. The amounts of O2 and CO2 are expressed by a cc/h-kg number. The first ten tests showed the normal metabolism, the second ten the metabolism during the Mg administration. The mean value in these tests remained considerably under the norm. The significant difference (K) calculated from these average values and the probable errors show actually a considerable difference, which exceeds 4 in all cases, mostly even 6. In five tests of the second group it could be shown that the value of the basic metabolism returned to normal again after the feeding of Mg was stopped.

III Sodium gluconate tests to test the acute effect

Basic metabolism tests made in connection with the administration of different sodium salts can be found in great number in the literature.

In one part of these tests an increase of the basic metabolism was observed, in another part a decrease, depending on which sodium compounds were used, and how, in what dose and how long they were given. An increase of the basic metabolism was observed by Loewy (1) and Raeder (2) after the administration of Na2CO2, by Loewy (3) after borax. The basic metabolism remained unchanged after peroral administration of Na2CO3 and NaCl; according to Leindoerfer (4) it diminishes after Na3PO4 and according to Henriques (5) after long-term intravenous injection of NaNO3 and Na2SO4. In tests of the tissue metabolism,

Myrhmann (6) observed on the frog leg a reduction of the oxidation after the administration of NaCl, KCl, CaCl2, MgCl2. Other scientists, on the other hand, found an increase of the basic metabolism after administration of the Na-salts.

The above mentioned results do not provide an answer, however, to the question which were raised by us. These tests were carried out everywhere with salts containing inorganic ions; we thus have every reason to assume that- apart from the size of the dose - the specific ion-effect of the anions plays a role in the results. For this reason we made sodium gluconate the subject of our tests, of which 0.05 mg per g body weight were injected in this series of our tests.

we used four rats each for our tests. In the first series we determined the basic metabolism of the four animals in two hour tests, then we continued to determine the basic metabolism, for another 6 hours after the injection of Na-gluconate. Our test results are compiled in table III.

According to these tests, the injection of Na-gluconate causes no major changes in the basic metabolism. In the animals no. 16 and 17 the difference is +7.5% and +7.3% resp. in animal no. 13 +3.1%, in no. 18 -1.6%. These differences are minor and not clear and can thus not be considered as a major change.

IV Sodium gluconate feeding tests

In this test series we determined the basic metabolism on four rats put on a normal diet, in a test series each of ten individual tests, adding to the diet Na-gluconate (0.05 mg Na per g and day) and we determined again in ten tests the basic metabolism in the course of the gluconate feeding; finally we determined the further behavior of the basic metabolism after the administration was stopped by making one test a week. According to the results compiled in table IV, the basic metabolism of the animals diminishes

in the course of the Na-feeding. The statistical evaluation verifies the accuracy of the results. A few weeks after the administration was stopped, the basic metabolism returned again to the normal value.

V. Gluconate control

In connection with the basic metabolism-reducing effect of magnesium- and sodium gluconate, the question arose whether the gluconate-ion did not play a role in the reduction of the metabolism. In order to answer this question, we made feeding control tests on two animals, with the same dose of gluconic acid which had been combined with sodium and magnesium in the preceding tests.

According to table V, gluconic acid does not cause a substantial change in the basic metabolism, which is also demonstrated by the low value of the significant difference.

It can therefore be concluded from the results of these tests that the change in the basic metabolism following the long term administration of magnesium- and sodium gluconate is not caused by the gluconate, but by the specific chemical action of the magnesium - and sodium ion.

Summary

1. After subcutaneous injection of Mg-gluconate, the O2-consumption in the rat diminishes in the first 2 hours by 20 - 40%
2. After this effect has worn out, the O2-consumption increases in the majority of animals by 5 - 20% above the normal value, in a smaller part the values increase only up to the norm.
3. The increase of the metabolism after a single injection of Mg-gluconate disappears completely after 6-7 hours.
4. After long-term peroral administration of Mg gluconate the basic metabolism of the rat diminishes significantly by 10-15%.

5. With the dose selected by us, 10-20 days are required until the effect appears, which continues then for about 10-20 days.
6. After subcutaneous injection of Na-gluconate the basic metabolism of rats does not show any substantial change.
7. After long-term peroral administration of Na-gluconate the basic metabolism diminishes significantly by 10 - 18%.
8. The reduction of the basic metabolism by sodium appears 2-3 weeks after the administration and lasts then for 2-3 weeks.
9. After long-term peroral administration of gluconic acid there is no substantial change in the basic metabolism.
10. The reduction of the basic metabolism following the effect of magnesium- or sodium gluconate is due to the action of the magnesium- and sodium ion.

(Translated by Carl Demrick Associates,Inc/IE/t)

Die Wirkung von Alkalimetall-Ionen auf den Grundstoffwechsel.

Von

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Befaßt man sich mit den Wirkungen der Heilwässer, so ergibt sich die Frage, welchen Einfluß diese Wässer auf den Grundstoffwechsel ausüben. Um ihre Gesamtwirkung zu erkennen, muß man zuerst die ihrer einzelnen Ionen gesondert erforschen. In dieser Arbeit berichten wir über unsere auf die Magnesium- und Natriumwirkung bezüglichen Untersuchungen.

Versuche, welche in unserem Institut durch *Kliebert* (7) ausgeführt wurden, beweisen, daß Kaninchen, welche mit einem magnesium- und natriumhaltigen Mineralwasser getränkt wurden, auf eine Adrenalininjektion mit einer Hyperglykämie geringeren Grades und kürzerer Dauer reagieren als die unbehandelten Kontrollen. Gleichzeitig wird die bei Verabreichung von Dextrose eintretende Zuckerbelastungskurve viel höher, was durch eine bessere Anreicherung von Glykogen in der Leber erklärt werden kann.

Auch *Frankes* (8) Versuche weisen auf Mg-gluconatwirkung Glykogenspeicherung in der Leber nach. Nach *Hazard* und *Vaille* (9) genügt eine kleine Menge Magnesium zur Verminderung des Blutzuckers. Dasselbe beweisen auch die Versuche von *Lang* und *Rajó* (10). *Kliebert* (7) erklärt die bei seinen Versuchen beobachtete Mg-Wirkung als eine Umstimmung des vegetativen Nervensystems in parasympathischer Richtung.

Auf Grund des Vorstehenden erschien es interessant, die Literatur von dem Gesichtspunkte aus zu durchsuchen, welchen Einfluß ein parasympathisches Übergewicht auf den Grundstoffwechsel ausübt. Nach *Lubbi* und *Rubinstein* (11) vermindert das parasympathicomimetische Acetylcholin und das Sympathicus lähmende Yohimbin den Grundstoffwechsel in gleicher Weise. Dieselben Autoren sahen auch beim Ergotamin eine ähnliche Wirkung. Nach *Orestano* (12) vermindert das Ergotamin ebenfalls den Grundstoffwechsel und mit Adrenalin zugleich gegeben verhindert es dessen erhöhende Wirkung. Alle diese Autoren stimmen in bezug auf die den Grundstoffwechsel steigernde Wirkung des Adrenalins überein und bezeichnen die Wirkung von Atropin und Pilocarpin als zweiphasisch.

Auf Grund der erwähnten Angaben müssen wir darauf schließen, daß bei Verabreichung von Magnesiumsalzen der Grundstoffwechsel vermindert wird, wenn das Magnesium wirklich eine in der parasympathischen Richtung erfolgende Verschiebung des vegetativen Gleichgewichts des Organismus hervorruft. In bezug auf die Na-Wirkung

Magnesium gluconate

sind uns ähnliche Vorarbeiten nicht bekannt. Wir verabreichten das Mg und Na in Gestalt ihrer Gluconsalze unter der Voraussetzung, daß das Gluconat als Anion sich indifferent verhält, und die Wirkung des Kations demzufolge möglichst ungestört zur Geltung kommt.

Die Versuche wurden in zwei Gruppen eingeteilt: in der ersten beobachteten wir die auf eine *einmalige Injektion* von Mg- bzw. Na-gluconat *sofort* eintretende Grundstoffwechselveränderung, in der zweiten dagegen die auf *längerdauernde Mg- bzw. Na-gluconatfütterung* eintretenden *späteren* Veränderungen.

Die Bestimmung des Grundstoffwechsels erfolgte zum Teil mit dem ursprünglichen Stoffwechselapparat für Kleintiere von *Belák-Illényi* (13), zum Teil mit der von *Berta* angegebenen Modifikation desselben, welche in der vorgehenden Arbeit genau beschrieben wurde.

Unter den zur Untersuchung verwendeten 15 Stück 160 bis 220 g schweren normal entwickelten, männlichen Ratten erwiesen sich zu Stoffwechselversuchen vier Tiere als vollkommen geeignet. An diesen führten wir zur Bestimmung des normalen Grundstoffwechsels nach 16—20stündigem Hungern dreimal einen 2 Stunden dauernden Versuch aus. Nach den vorgenommenen statistischen Berechnungen waren die Abweichungen vom Mittel sehr gering — die Fehler betrugen $^1/_{100}$ oder $^1/_{200}$ des Durchschnittswertes —, so daß die Zuverlässigkeit unserer Mittelwerte außer allem Zweifel steht.

Um zu vermeiden, daß die saisonmäßigen Veränderungen der Fütterung den Grundstoffwechsel unserer Ratten beeinflussen, hielten wir sie auf einer gleichbleibenden Diät, welche aus einem Weizen-, Gerste- und Maisgemisch, außerdem aus Casein und Fett bestand. Zur Deckung des Vitaminbedarfs enthielt dieselbe Butter und Hefe, als erforderliche Salzmenge das Salzgemisch nach *Mc. Collum*. Die Versuche führten wir stets mit 16—20 Stunden lang hungernden Tieren aus.

I. Magnesium-Versuche zur Prüfung der akuten Wirkung.

In der nachstehenden Versuchsreihe injizierten wir 1,5—2,0 ccm 10%ige Magnesiumgluconatlösung subcutan und bestimmten nach deren Verabreichung 6 Stunden hindurch in $^1/_2$stündigen Intervallen den Grundstoffwechsel des Tieres. Bei den Ratten Nr. 5, 10, 13 führten wir je drei, bei Nr. 7 wegen Verenden des Tieres bloß einen Versuch in dieser Weise aus.

Auf die Injektion von Mg-gluconat verringert sich der Grundstoffwechsel der Ratten (Tabelle I). Diese Verminderung erreicht ihren Tiefpunkt in der 3.—4. halben Stunde, in der nämlich — den normalen O_2-Verbrauch mit 100% angenommen — dieser auf 53—83% absinkt, so daß wir eine Senkung von 17—47% beobachten können. Diese Verringerung trat in jedem Versuche ein. Darauf beginnt der Grundstoffwechsel wieder zu steigen und erreicht in der 5.—8. halben Stunde

Tabelle I.

Tier Nr.	Mittel aus je drei Normalversuchen	Tag des Versuchs	O_2-Verbrauch nach einer Injektion in den einander folgenden halben Stunden. (Mg-Gluconat)													
			0—½	½—1	1—1½	1½—2	2—2½	2½—3	3—3½	3½—4	4—4½	4½—5	5—5½	5½—6	6—6½	6½—7
5	1214	20. I.	--	920	849	769	832	888	1045	1126	1250	1250	1222	1200	—	—
		15. III.	1219	1167	887	834	1015	966	1126	1207	1279	1247	1439	1117	1125	1216
		20. III.	1151	986	1015	936	985	1133	1231	1329	1230	1211	1196	1221	—	—
7	1208	12. III.	—	—	751	1017	1090	1110	1061	1092	1092	1171	1171	1207	1225	—
10	1016	1. III.	1109	883	971	971	971	1022	1142	1020	1011	1079	1021	1009	—	—
		11. III.	—	784	711	727	818	940	953	998	1030	1037	935	981	1006	1020
		18. III.	--	956	759	893	1076	1076	1076	1203	1016	1203	1016	1016	—	--
13	1071	22. I.	1016	997	840	962	918	918	1037	1000	1164	1174	1062	1052	1075	1075
		4. III.	—	659	612	879	1125	1232	1201	1179	912	1089	1089	—	—	—
		16. III.	1065	1024	888	898	961	1138	1107	1190	1304	1138	1093	1093	—	—
M			1118	931	823	883	979	1045	1098	1134	1129	1159	1124	1099	1108	1103
$P.E._M$			28,6	32,8	21,1	18,6	19,7	24,6	14,4	22,8	28,8	15,5	30,1	20,9	31.	39,4
K			0,2	5,1	10,4	9,2	5,4	2,6	1,1	0,3	0,1	1,4	0,03	0,9	0,5	0,05

M = Mittelwert.
$P.E._M$ = Wahrscheinlicher Fehler des Mittelwertes.
K = Signifikante Differenz des Mittelwertes.

das normale Niveau, manchmal erhebt er sich sogar noch darüber um sich dann nach kleineren oder größeren Schwankungen auf die normale Höhe einzustellen; in einem anderen Teile der Fälle verbleibt er ständig auf dem normalen Niveau.

Die in dieser Versuchsreihe angewandte Mg-gluconatlösung kann in osmotischer Beziehung ungefähr als physiologische Lösung angesehen werden. Infolge ihrer allgemeinen physikalischen Wirkung lassen solche Salzlösungen den Grundstoffwechsel entweder unverändert oder erhöhen ihn in geringem Maße. Eine Beobachtung, nach welcher der Grundstoffwechsel physikalisch verkleinert wird, ist im Schrifttum überhaupt nicht vorzufinden, weshalb unser Befund nur von der chemischen Wirkung des Mg-gluconats herrühren kann. Das Gluconat ist jedoch, wie dies auch unsere später mitgeteilten Versuche beweisen, als organisches Anion ebenfalls unwirksam, so daß die Grundstoffwechselverminderung der Mg-Wirkung zuzuschreiben ist.

Im Laufe unserer Versuche tauchte noch der Gedanke auf, ob die hier beobachtete Wirkung nicht bloß die Folge der narkotischen, richtiger einschläfernden, beruhigenden Wirkung des Magnesiums sei; dies ist jedoch schon darum nicht wahrscheinlich, weil nach unseren Beobachtungen die Beweglichkeit der Ratten nach Einspritzung des Magnesiums sich nicht änderte. Wir wollen erwähnen, daß nach *Matthei* und *Butturini* (14) beim Kaninchen wenigstens 5 g/kg $MgSO_4$ eine Narkose hervorruft. Diese Menge entspricht, auf reines Mg umgerechnet, ungefähr 0,6 g, demgegenüber haben wir nur 0,05 g Mg angewandt. Von dieser kleinen Dosis ist also eine narkotische Wirkung nicht anzunehmen. Bezüglich der auf den Grundstoffwechsel ausgeübten Wirkung der Narkotica wären noch *Lee Miltons* (15) Versuche zu erwähnen, nach denen 0,05 g/kg Isoamyläthylbarbitursäure — also eine sehr stark betäubende Dosis — eine 5—15% Verminderung hervorruft, 0,60—0,90 g/kg Urethan, also ebenfalls eine große Dosis, nur eine solche von weniger als 10%. Die in unseren Versuchen beobachtete Verminderung des Grundstoffwechsels kann daher mit der narkotischen Wirkung des Mg nicht erklärt werden.

In ähnlichem Sinne sind auch die Versuche von *Tangl* und *Verzár* (16) zu verwerten, welche fanden, daß der Grundstoffwechsel durch Curare nur bei solchen Tieren gesenkt werden kann, die an Stoffwechselbestimmungen nicht gewöhnt waren, bei daran gewöhnten jedoch nicht. Unsere Tiere, welche eine große Zahl von Versuchen durchmachten, sind also als angewöhnt zu betrachten.

II. Magnesium-Fütterungsversuche.

In der zweiten Gruppe unserer Versuche prüften wir die auf Grund dauernder Mg-Verabreichung eintretende Veränderung des Grundstoffwechsels.

Für unsere Versuche wählten wir 20 Ratten. Im allgemeinen bestimmten wir je zehnmal in zweistündiger Untersuchungsdauer den normalen Grundstoffwechsel der Tiere und wählten zum Hauptversuche jene vier Tiere aus, bei denen der wahrscheinliche Fehler (P. E.) des Mittelwertes (M) kleiner als 2% desselben war. Der Stoffwechsel solcher Tiere ist genügend gleichmäßig, um die Abweichung vom normalen Wert verläßlich beobachten zu können. Während der ganzen Versuchsreihe hielten wir die Tiere auf der *Mc. Collum*schen Normaldiät, welche wir genau dosierten, so daß die Tiere täglich 7,5% ihres Körpergewichts erhielten. Nach Feststellung des normalen Grundstoffwechsels wurden die Tiere mit Mg gefüttert und ihr Grundumsatz wieder bestimmt. Zu diesem Zwecke führten wir neuerlich zehn Versuche durch. Nach Ausführung von zehn Versuchen stellten wir die Mg-Darreichung ein und beobachteten, wöchentlich je eine Bestimmung vornehmend, noch durch 5 Wochen die Rückkehr des Grundstoffwechsels zum normalen Wert.

Zu unseren Versuchen wählten wir als Untersuchungsmaterial eine Mg-gluconatlösung, welche pro ccm 5 mg reines Mg enthielt; die Ratten bekamen dann pro g Körpergewicht täglich 0,01 ccm in die Normaldiät gemischt, bei der wir den ursprünglichen Grundstoffwechsel bestimmt hatten. Auf diese Weise wurden also täglich pro g Körpergewicht 0,05 mg reines Mg verabreicht.

Die Bestimmungen führten wir in vollkommen gleicher Weise wie oben aus. Die Ergebnisse unserer Untersuchungen sind in Tabelle II angeführt. Die O_2- und CO_2-Menge drückten wir durch die ccm/Stunden-kg-Zahl aus. Die ersten zehn Versuche zeigen den normalen Stoffwechsel, die zweiten zehn den Stoffwechsel während der Mg-Verabfolgung. Der Mittelwert bei diesen Versuchen blieb bedeutend unter dem Normalen. Die aus diesen Durchschnittszahlen und den wahrscheinlichen Fehlern errechnete signifikante Differenz (K) zeigt tatsächlich einen bedeutenden Unterschied, dessen Wert überall über 4, meistens sogar über 6 beträgt. In je fünf Versuchen der dritten Versuchsgruppe konnte nachgewiesen werden, daß nach Einstellung der Mg-Verabfolgung der Wert des Grundstoffwechsels wieder zur Norm zurückkehrt.

III. Natriumgluconatversuche zur Prüfung der akuten Wirkung.

In Zusammenhang mit der Verabreichung von verschiedenen Natriumsalzen ausgeführte Grundstoffwechseluntersuchungen sind in der Literatur in großer Zahl zu finden.

In einem Teile dieser Untersuchungen wurde eine Erhöhung, in einem anderen eine Verminderung des Grundstoffwechsels beobachtet, je nachdem, welche Natriumverbindungen, in welcher Weise, in welcher Dosis und wie lange sie angewandt wurden. Eine Erhöhung des Grundstoffwechsels beob-

Tabelle II.

Tier Nr. / Versuchs-Nr.		5		6		12		20	
		O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
1	Normalwerte	1182	953	1218	969	1134	999	1266	944
2		1248	945	1278	904	1152	826	1272	892
3		1410	1012	1056	959	1188	929	1242	961
4		1200	908	1068	897	1236	1116	1176	857
5		1264	1187	1248	977	1092	953	1152	984
6		1182	910	1188	920	1116	889	—	—
7		1368	1198	1188	956	—	—	—	—
8		1242	955	1242	825	—	—	—	—
9		1134	846	1264	851	—	—	—	—
10		1266	1006	1272	972	—	—	—	—
Mittelwert: M_1 ...		1249	991	1201	924	1153	952	1221	927
Wahrscheinlicher Fehler: P.E.$_{M_1}$ ..		17,8	24,2	16,8	11,6	14,3	27,4	16,4	14,6
1	Während der Mg-gluconat-fütterung	1248	890	1212	865	1158	811	1224	907
2		1110	864	1140	839	1146	799	1104	869
3		1164	906	1014	771	1062	780	1002	738
4		1092	796	1074	846	984	699	1140	796
5		1092	805	1008	791	924	735	984	693
6		1164	880	1050	821	999	749	918	681
7		1158	923	1008	777	990	776	1056	753
8		1242	924	1056	775	942	752	1014	659
9		1080	866	1062	786	1116	824	1104	850
10		1062	811	1026	804	900	710	1098	796
M_2		1141	866	1065	808	1022	764	1064	776
P.E.$_{M_2}$		14,16	15,87	19,56	7,13	19,8	8,92	18,84	17,91
K		4,24	4,33	6,6	8,52	5,36	6,51	6,25	6,58
1	Nach der Mg-gluconat-fütterung	1116	822	1276	901	1105	778	1201	883
2		1300	933	1318	803	1225	1048	1324	980
3		1368	970	1457	991	1034	814	1254	986
4		1343	1028	1084	777	1221	1004	1221	879
5		1133	889	1076	838	1020	809	1020	809
M_3		1252	928	1201	862	1121	890	1264	907

achteten *Loewy* (1) und *Räder* (2) auf die Wirkung von Na_2CO_3, *Loewy* (3) auf Boraxwirkung. Unverändert bleibt der Grundstoffwechsel auf perorale Verabreichung von Na_2CO_3 und NaCl, nach *Leimdörfer* (4) vermindert er sich auf Na_3PO_4 und nach *Henriques* (5) auf dauernde intravenöse Verabfolgung von $NaNO_3$ und Na_2SO_4. In Gewebsstoffwechseluntersuchungen beobachtete *Myrhmann* (6) am Froschschenkel auf die Wirkung von NaCl, KCl, $CaCl_2$, $MgCl_2$ eine Verminderung der Oxydation. Ihm gegenüber fanden andere Untersucher auf Wirkung der Na-Salze eine Erhöhung des Grundstoffwechsels.

Die erwähnten Ergebnisse geben jedoch keine Antwort auf die Frage, die wir uns vorgelegt hatten. Diese Versuche wurden überall mit anorganische Anionen enthaltenden Salzen ausgeführt; wir haben also alle Ursache anzunehmen, daß in die Ergebnisse — abgesehen von der Größe der Dosis — auch die spezifische Ionenwirkung der Anionen hineinspielt. Deshalb machten wir zum Gegenstande unserer Untersuchung das Natriumgluconat, von dem wir in dieser Reihe unserer Versuche pro g Körpergewicht 0,05 mg injizierten.

Zu unseren Versuchen verwandten wir je vier Ratten. In der ersten Versuchsreihe bestimmten wir den Grundstoffwechsel der vier Tiere in zweistündigen Versuchen, dann führten wir nach der Injektion von Na-gluconat die Grundstoffwechselbestimmungen durch weitere 6 Stunden aus. Unsere Versuchsergebnisse stellen wir in Tabelle III zusammen.

Nach diesen Versuchen bringt die Na-gluconatinjektion im Grundstoffwechsel keine größeren Veränderungen zustande. Bei den Tieren Nr. 16 und 17 beträgt der Unterschied + 7.5% bzw. + 7.3%, beim Tier Nr. 13 + 3.1%, bei Nr. 18 — 1.6%. Diese Unterschiede sind geringfügig und nicht eindeutig, also nicht als bedeutende Veränderung zu betrachten.

IV. Natriumgluconat-Fütterungsversuche.

In dieser Versuchsreihe bestimmten wir bei vier auf normale Diät gesetzten Tieren den Grundstoffwechsel in je einer Versuchsreihe von zehn einzelnen Versuchen, fügten danach zur Diät Na-gluconat (0,05 mg Natrium pro g und Tag) hinzu und bestimmten in neuerlichen zehn Versuchen im Laufe der Gluconatfütterung den Grundstoffwechsel; endlich stellten wir nach Beendigung der Verabfolgung mittels je einer Untersuchung wöchentlich das weitere Verhalten des Grundstoffwechsels fest. Nach den in der Tabelle IV angeführten Ergebnissen vermindert sich der Grundstoffwechsel der Tiere im Laufe der Na-Fütterung. Die statistische Auswertung sichert die Richtigkeit der Ergebnisse. Einige Wochen nach Einstellung der Verabreichung kehrte der Grundstoffwechsel wieder auf den normalen Wert zurück.

Tabelle III.

Tier Nr.		Normalwerte		Nach der Natriumgluconat-Injektion in den einander folgenden Stunden					
		1. Std.	2. Std.	0—1	1—2	2—3	3—4	4—5	5—6
13	O_2	1130	1144	1190	1152	1079	1067	1080	1148
	CO_2	861	847	877	829	754	757	756	794
	O_2	1135	1124	1148	1184	1150	1068	1180	1160
	CO_2	805	806	815	835	818	756	844	814
	O_2	1108	1128	1153	1115	1190	1125	1114	1134
	CO_2	777	811	834	799	785	801	774	793
16	O_2	1238	1253	1257	1246	1378	1198	1312	1359
	CO_2	899	899	899	901	978	837	909	978
	O_2	1207	1218	1330	1293	1271	1217	1256	1279
	CO_2	907	936	983	957	905	875	906	914
	O_2	1127	1111	1431	1375	1320	1333	1323	1265
	CO_2	834	849	1017	906	973	1000	1040	897
17	O_2	1043	1008	1009	1014	1047	1005	1046	1047
	CO_2	767	726	700	762	757	735	742	714
	O_2	1003	1009	1065	1025	1022	991	1030	1028
	CO_2	693	726	757	740	716	738	730	739
	O_2	1061	1066	1047	1114	1097	1127	1254	1124
	CO_2	795	792	754	793	784	779	844	798
18	O_2	1188	1233	1156	1191	1188	1220	1144	1218
	CO_2	849	846	840	834	822	831	816	828
	O_2	1028	1092	1059	1057	1103	1169	1123	1110
	CO_2	801	775	816	772	803	832	804	879
	O_2	1136	1071	1192	1062	1049	1109	1087	1112
	CO_2	807	773	777	754	747	776	783	811
M....	O_2	1119		1161	1152	1157	1138	1162	1165
	CO_2	816		836	823	820	810	829	830
P.E$_M$.	O_2	10,8		23,9	21,6	22,3	19,5	17	18,8
	CO_2	8,0		13,3	13,3	15,3	14,5	17,1	14,6
K....	O_2			1,6	1,4	1,5	0,9	2,1	2,1
	CO_2			1,3	0,4	0,2	0,4	0,7	0,8

V. Gluconatkontrolle.

Im Zusammenhang mit der den Grundstoffwechsel vermindernden Wirkung des Magnesium- bzw. Natriumgluconats ergab sich die Frage, ob bei der Verringerung des Stoffwechsels nicht auch das Gluconat-Ion mitspiele. Zur Feststellung dieser Frage führten wir an zwei Tieren Fütterungs-Kontrollversuche aus, und zwar mit derselben Gluconsäuredosis, welche in den vorstehenden Untersuchungen an Magnesium bzw. Natrium gebunden war.

Tabelle IV.

Tier Nr. / Versuchs-Nr.		15		16		17		18	
		O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
I.	Normalwerte	894	721	1266	1011	1296	959	1320	1118
II.		1134	780	1182	1158	1260	989	1104	733
III.		1218	981	1250	960	1104	703	1236	824
IV.		1026	931	1146	1019	1098	786	1116	1093
V.		1122	778	1332	1275	1098	814	1218	956
VI.		1104	852	1320	891	1068	757	1206	831
VII.		1062	802	1266	994	1098	768	1104	821
VIII.		1038	756	1218	963	1080	822	1116	770
IX.		1110	836	1254	929	1122	818	1164	893
X.		1080	822	1278	921	1104	835	1218	869
M_1		1080	826	1261	1012	1127	834	1181	882
P. E.$_{M_1}$		16,6	16,9	13,9	23,22	21,0	18,33	14,8	24,16
I.	Während der Na-gluconat-fütterung	999	754	1054	805	870	673	932	716
II.		1018	800	1154	842	957	772	1091	819
III.		1017	800	1089	905	864	674	994	891
IV.		824	630	1176	854	958	692	1011	700
V.		1004	765	1092	842	1081	867	832	610
VI.		976	720	956	753	826	590	954	730
VII.		990	696	1003	686	1006	757	969	776
VIII.		841	597	941	661	715	466	1050	822
IX.		892	707	936	690	1161	752	1104	790
X.		883	688	1008	766	826	541	884	549
M_2		944	716	1041	780	928	678	982	746
P. E.$_{M_2}$		16,2	11,7	18,8	17,35	25	25,6	17,4	21,7
K		**5,51**	**5,35**	**9,55**	**8,05**	**6,95**	**4,95**	**8,71**	**4,20**
I.	Nach der Na-gluconatfütterung	864	612	1110	872	946	700	983	718
II.		875	581	1249	884	966	755	1168	791
III.		1322	978	1329	966	1085	795	1230	956
IV.		1030	705	1186	892	1047	773	1065	781
V.		886	665	1031	787	1018	731	1075	800

Tabelle V.

Tier Nr. / Versuchs-Nr.		♂ O_2	♂ CO_2	♀ O_2	♀ CO_2
I.	Normalwerte	1169	900	1083	75·
II.		1148	870	1048	740
III.		1176	890	990	820
IV.		1102	825	1012	740
V.		1140	780	1045	828
VI.		1190	910	1114	754
VII.		1054	959	993	718
VIII.		1158	920	1090	780
IX.		1145	910	999	893
X.		1163	890	1041	761
M_1		1147	885	1041	778
P. E.$_{M_1}$		7,86	10,8	9,17	11,6
I.	Während der Gluconsäurefütterung	1071	788	854	682
II.		1214	1012	967	809
III.		1043	818	1074	755
IV.		1085	817	993	689
V.		1029	760	984	741
VI.		1263	984	1178	929
VII.		1140	844	1182	861
VIII.		1187	929	1150	825
IX.		1005	859	1072	732
X.		1110	850	1047	736
M_2		1121	866	1050	775
P. E.$_{M_2}$		16,3	21,3	22,2	16,6
K		1,4	0,79	0,37	0,15

Nach Tabelle V bringt die Gluconsäure keine wesentliche Veränderung im Grundstoffwechsel zustande, was auch der geringe Wert der signifikanten Differenz beweist.

Aus den Ergebnissen dieser Versuche kann daher geschlossen werden, daß die auf dauernde Verabfolgung von Magnesium- bzw. Natriumgluconat eintretende Grundstoffwechselverminderung nicht dem Gluconat, sondern der spezifisch chemischen Wirkung des Magnesium- bzw. Natrium-Ions zuzuschreiben ist.

Zusammenfassung.

1. Auf subcutane Injektion von Mg-gluconat vermindert sich bei der Ratte der O_2-Verbrauch in den ersten 2 Stunden um 20—40%.

2. Nach dem Abklingen dieser Wirkung tritt im O_2-Verbrauch der Tiere in der Mehrzahl der Fälle eine Erhöhung von 5—20% über den normalen Wert ein, in einem kleineren Teile der Fälle steigen die Werte nur bis zur Norm.

3. Die durch eine einmalige Injektion des Mg-gluconats eintretende Stoffwechselsteigerung klingt in 6—7 Stunden vollständig ab.

4. Bei dauernder peroraler Verabreichung von Mg-gluconat sinkt der Grundstoffwechsel der Ratten signifikant um 10—15%.

5. Bis zum Eintreten der Wirkung sind im Falle der von uns gewählten Dosis 10—20 Tage erforderlich, dieselbe hält dann ungefähr 10—20 Tage an.

6. Auf subcutane Injektion von Na-gluconat zeigt der Grundstoffwechsel von Ratten keine wesentliche Veränderung.

7. Auf dauernde perorale Verabfolgung von Na-gluconat vermindert sich der Grundstoffwechsel signifikant um 10—18%.

8. Die Stoffwechselverminderung des Natriums tritt 2—3 Wochen nach der Verabfolgung ein und dauert dann noch 2—3 Wochen an.

9. Auf dauernde perorale Verabreichung von Gluconsäure kommt keine wesentliche Stoffwechselveränderung zustande.

10. Die auf die Wirkung des Magnesium- bzw. Natriumgluconats eintretende Grundstoffwechselverminderung ist der Wirkung des Magnesium- bzw. Natrium-Ions zuzuschreiben.

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Biochemical Journal

C. THE EFFECTS OF THE INJECTION OF CERTAIN SALTS ON THE CALCIUM, MAGNESIUM AND INORGANIC PHOSPHORUS OF THE SERUM OF THE RABBIT.

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BROOKFIELD [1933] showed that in the serum of the rabbit there was an inverse relationship between calcium and magnesium, a direct relationship between magnesium and inorganic phosphorus and an inverse relationship between calcium and inorganic phosphorus, the latter of which had been previously observed by Dupré and Semenoff [1931] and Bourne and Campbell [1932]. The rises and falls in the concentrations of the three ions in the serum were correlated with their relative amounts in the diet. The relationships seemed worthy of further study under other experimental conditions, in order to determine whether the effects observed with diets varying in their mineral contents were of more general significance, and accordingly another series of experiments has been carried out.

Solutions of salts containing Ca^{++}, Mg^{++} and $PO_4^{=}$ ions have been injected subcutaneously into rabbits and their effects on the calcium, magnesium and inorganic phosphorus of the serum observed.

EXPERIMENTAL.

Experiments were made on fully grown male rabbits maintained on a diet of cabbage and oats, which (with one exception) had fasted for about 18 hours. Calcium gluconate, laevulate and lactate, magnesium sulphate and lactate and disodium hydrogen phosphate were the salts employed, while control experiments were carried out with sodium chloride and sodium sulphate. The salts, dissolved in the minimum volume of water, were injected subcutaneously into the flank. 5 ml. of blood were withdrawn from the lateral vessel of the ear immediately before the injection and on three separate occasions afterwards. The effects were observed over periods varying from 1½ to 6 hours. The methods of estimating calcium, magnesium and inorganic phosphorus were the same as those used in the dietary experiments [Brookfield, 1933].

The influence of two factors called for control before the results of the experiments could be interpreted; firstly, the effects of four small haemorrhages recurring at relatively short intervals and secondly, the effects of the injection of a solution of an indifferent salt.

1. *The effects of bleeding.* (*a*) *Successive small haemorrhages.* 5 ml. of blood were removed from a rabbit four times at half-hourly intervals; 9 days later the same procedure was repeated, but at hourly intervals and after a further 22 days the animal was bled again, this time at 2-hourly intervals. In the

first experiment the haemoglobin percentage was unaltered at the fourth bleeding: in the other two experiments a fall of 8 % was noted. The serum-calcium, magnesium and inorganic phosphorus values are recorded in Table I.

Table I. *Serum-calcium, magnesium and inorganic phosphorus. Small haemorrhages at short intervals.*

Rabbit 20. Weight 2010 g. 4 bleedings of 5 ml. on each occasion.

Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
½-hourly intervals		
16·55	1·952	3·152
15·70	1·694	2·626
15·45	1·597	2·551
14·95	1·590	2·716
1-hourly intervals		
13·29	2·886	4·382
13·29	2·886	4·382
12·94	2·794	4·762
12·72	2·749	4·794
2-hourly intervals		
13·53	3·615	3·750
13·53	3·542	3·936
13·90	3·357	4·521
13·67	3·204	4·654

It will be seen that the magnesium value tends to fall, but at a very slow rate. The calcium figures show variable results, depending on the time interval. The four bleedings at half-hourly intervals have caused a fall of 1·6 mg. (10 %). Hourly bleedings cause a slighter fall (4 %) and 2-hourly bleedings none. A fall in the inorganic phosphorus followed by a rise after one hour results from bleeding at intervals of half an hour, while with hourly or 2-hourly bleedings the inorganic phosphorus rises.

(*b*) *Progressive changes during a single haemorrhage.* The rapidity with which the fall in the serum-calcium takes place is illustrated by the results recorded in Table II.

Table II. *Serum-calcium and magnesium values during bleeding. Blood taken in two samples of 5 ml. each.*

Rabbit no.	Time of bleeding	Calcium mg./100 ml.	Magnesium mg./100 ml.
19	11.45–12.15	15·28	1·722
	12.15–12.45	14·44	1·694
21	4.30–4.45	15·73	1·895
	4.45–5.0	15·35	1·886
19	10.45–11.0	12·60	1·907
	11.0–11.10	12·25	1·896
20	11.15–11.25	14·35	1·963
	11.25–11.30	14·25	1·951

Each animal was submitted to a single haemorrhage and the blood collected in two successive 5 ml. samples. In one experiment where the time of bleeding was rather prolonged, a fall of 0·8 mg. (6 %) was noted. The effects of haemorrhages on the serum-calcium of rabbits have been referred to by several writers. A decrease of 10 % or more has been reported when observations have been made

at a short interval after the haemorrhage by Stransky [1915], Clark [1920], Stewart and Percival [1927] and Culhane [1927]. Seven hours after bleeding, Moritz [1925] found a slight and inconstant decrease in the serum-calcium value. A fall in the magnesium also was noted by Stransky. These results show that the variations in the concentrations of calcium, magnesium and inorganic phosphorus caused by bleeding are so slight as to have little or no significance in the interpretation of the findings in the injection experiments, which will now be discussed.

2. *The effects of the injection of an indifferent salt.* Sodium chloride was injected in an amount somewhat in excess of those of the other salts employed. The results are recorded in Table III.

Table III. *Injection of sodium chloride. Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 24. Weight 2300 g. 5 ml. bleedings. Fasting.

Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	13·29	2·786	3·820
0·36 g. NaCl (0·0062 g. equivalent per kg. in 9 ml. water injected subcutaneously).			
½	13·29	2·806	4·154
1	12·67	2·726	3·434
4	13·06	2·557	3·701

There is a slight fall in all three constituents after one hour with a tendency on the part of the calcium and inorganic phosphorus to return to their initial values after 4 hours. These changes are unimportant in comparison with the results which follow, and they show that probably any effect observed in the injection of salts containing Ca^{++}, Mg^{++}, or PO_4 was to be attributed to these ions themselves.

3. *The injection of solutions containing the Ca^{++}, Mg^{++}, PO_4 and SO_4 ions.* An attempt was made, in each case, to introduce an amount of salt sufficient to cause a marked effect on the several blood-constituents under review without producing untoward symptoms. The success achieved was only partial. No symptoms were noted after the injection of the calcium salts. Slight ataxia of the hind-limbs resulted from the injection of magnesium sulphate and lactate. Definitely toxic effects were observed however with disodium phosphate and sodium sulphate. In both cases there was some degree of collapse after the injection. The animal injected with disodium phosphate showed marked muscular twitching but ultimately recovered. The animal which received sodium sulphate was found dead 2 days later. No urine was passed during the period of any of the experiments except by the rabbit which received sodium sulphate. None of the animals suffered from diarrhoea.

Results.

For purposes of comparison the amounts of the salts have been expressed as g. equivalents per kg. body weight. The results of the injections of the calcium salts are set out in Table IV, of the magnesium salts in Table V, of sodium sulphate in Table VI and of disodium phosphate in Table VII.

1. *The interrelations of calcium and magnesium.* In each of the 4 experiments where calcium salts have been injected approximately equivalent amounts of calcium have been given. A moderate increase in the serum-calcium has been the result amounting on the average to 3·0 mg. or 23 % of the initial value.

The rise to the maximum takes place within one hour and is followed by an almost equally rapid fall. The rise in the serum-calcium is accompanied by a progressive fall in the serum-magnesium. In the case of the injection of calcium gluconate, there may be a slow return towards normal at 6 hours. The average fall amounts to 1·0 mg. or 37 % of the initial value though it should be noted that in 3 of the experiments (Table IV) the fall appears still to be continuing at the time of the final observation.

Table IV. *Injection of calcium salts. Serum-calcium, magnesium and inorganic phosphorus values.*

	Salt injected	Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
Rabbit 19. 2520 g. Fasting	Gluconate (0·6 g. = 0·0028 g. equiv. Ca/kg.)	0	13·17	2·389	3·720
		½	16·13	1·896	3·861
		2	14·91	1·590	4·106
		6	14·32	1·748	4·152
Rabbit 23. 2000 g. Fasting	Gluconate (0·75 g. = 0·0035 g. equiv. Ca/kg.)	0	13·29	3·091	4·826
		½	16·59	2·567	4·700
		1	16·75	2·097	4·609
		1½	17·77	1·896	4·700
Rabbit 23. 2200 g. Fasting	Lævulate (0·6 g. = 0·0039 g. equiv. Ca/kg.)	0	12·27	3·014	4·252
		½	15·19	2·635	3·990
		1½	14·57	2·136	—
		2¼	13·48	1·860	—
Rabbit 23. 2170 g. Fasting	Lactate (0·46 g. = 0·003 g. equiv. Ca/kg.)	0	14·46	2·332	2·977
		½	17·02	1·902	3·106
		1	14·97	1·620	2·927
		2	14·82	1·544	3·247

Though the magnesium salts have been injected in amounts equivalent to those of calcium the effects have been considerably greater. The rise in the serum-magnesium averages 5·6 mg. over the initial value, as compared with a rise of 3·0 mg. in the calcium value caused by injections of corresponding amounts of calcium salts. This rise takes place rapidly but is more sustained than in the case of calcium, and it is clear that either the rate of absorption of magnesium salts is much less rapid than that of calcium salts, or alternatively and more probably

Table V. *Injection of magnesium salts. Serum-calcium, magnesium and inorganic phosphorus values.*

	Salt injected	Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
Rabbit 19. 2680 g. Fed with cabbage and oats	Sulphate (0·37 g. = 0·003 g. equiv. Mg/kg.)	0	14·69	2·109	2·779
		¾	11·32	8·078	2·080
		1¼	10·74	8·258	1·890
		4¾	13·31	3·634	3·086
Rabbit 25. 2750 g. Fasting	Lactate (0·37 g. = 0·0031 g. equiv. Mg/kg.)	0	12·62	2·318	3·682
		¾	11·85	6·726	3·262
		1¾	10·51	7·466	3·147
		5	10·61	5·833	3·608

that the removal of excess magnesium from blood is much less readily accomplished. Where magnesium sulphate has been injected (Table V) the serum-magnesium is approaching the normal value after 5 hours, but at the same interval after the injection of the equivalent amount of magnesium lactate (Table V) the serum-magnesium is nearly 3 times greater than the initial value.

These increases in the magnesium concentration have resulted in a marked fall in the calcium value in every experiment. This fall in calcium has been more rapid and greater in extent (4·0 mg. or 27 % of the initial value), following the injection of magnesium sulphate (Table V). The calcium value in this experiment is approaching the initial level after 6 hours. Following the injection of magnesium lactate the serum-magnesium continues at a high level for a considerable time and there is a correspondingly prolonged depression of the serum-calcium, which after 5 hours is still at the low level of 10·6 mg.

The inverse relationship found to exist between calcium and magnesium in the dietary experiments is therefore borne out by the results of these subcutaneous injections of calcium and magnesium salts.

Previous observations of the effects of the injection of calcium and magnesium salts on the calcium and magnesium content of serum are fragmentary and in some cases conflicting. Salvesen *et al.* [1924, 2] found that the magnesium content of the blood of dogs was depressed by the intravenous injection of calcium chloride. Stransky [1915] and Meneghetti [1927] observed a fall in the serum-calcium of rabbits after the injection of magnesium sulphate, the latter worker also showing that the diffusible calcium was increased. A number of observers have shown that the injection of magnesium salts leads to an increase in the calcium excretion and *vice versa*. Thus Mendel and Benedict [1909] found that the injection of calcium chloride into dogs and rabbits led to loss of magnesium, while the injection of magnesium sulphate and chloride increased the excretion of calcium. Greenwald and Gross [1925] noted a temporary increase in the excretion of magnesium in dogs injected with calcium chloride. After introducing metallic magnesium under the skin of rabbits, Reding and Slosse [1923] noted a considerable increase in the output of calcium, and loss of calcium was also observed by Schiff [1920] and by Schiff and Stransky [1920] in infants injected with magnesium sulphate. While a fall in the serum-calcium, accompanied by an increased excretion of calcium, was noted by Pribl [1929] in rabbits after the injection of magnesium sulphate, a rise in the serum-calcium value with calcium retention following the injection of magnesium lactate was reported. Condorelli's [1926] findings are also at variance with those recorded above, in that he observed a considerable elevation of the serum-magnesium following the injection of calcium lactate into rabbits.

2. *The effects of the sulphate ion.* One possible complicating effect in the experiment with magnesium sulphate is the presence of the sulphate ion, for the fall in the calcium value was less when magnesium was introduced as the lactate. Since the injection of phosphate depresses the serum-calcium it was possible that the injection of sulphate might have had a similar effect, and this was controlled in an experiment in which sodium sulphate was injected in amount equivalent to twice that of the magnesium sulphate used. Table VI shows that this has

Table VI. *Injection of sodium sulphate (1·0 g. Na_2SO_4, $10H_2O \equiv 0{\cdot}006$ g. equiv. Na/kg.). Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 23. Weight 2500 g. Fasting.

Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	14·34	2·629	4·700
½	13·52	2·629	3·536
1¾	12·20	2·505	3·106
4	11·20	2·557	3·572

resulted in a considerable fall in the serum-calcium but one different in character from that caused by the injection of magnesium sulphate. The fall is much more gradual, and although still continuing at the time of the last observation, 4 hours after the injection, is less than that produced by half the equivalent amount of magnesium sulphate (Table V). It is of interest that Stransky [1915] found a much increased calcium excretion after the injection of sodium sulphate into a rabbit. Table VI shows that while sodium sulphate has no effect on the serum-magnesium, the serum-inorganic phosphorus is depressed by 1·6 mg. (34 %) to rise again after one and three quarter hours, when it moves in a direction opposite to that of calcium.

3. *The interrelations of calcium and inorganic phosphorus.* The changes in the serum-inorganic phosphorus after the injection of the three calcium salts (Table IV) are so slight that it appears that injected calcium has no direct effect on the inorganic phosphorus. When, however, disodium phosphate is injected there is an immediate fall in the serum-calcium (Table VII). In this experiment the phos-

Table VII. *Injection of disodium hydrogen phosphate (0·57 g. Na_2HPO_4, $12H_2O \equiv$ 0·0047 g. equiv. P/kg.). Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 19. Weight 2300 g. 5 ml. bleedings. Fasting.

Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	12·97	2·529	4·121
1	11·39	2·308	10·01
2	10·74	2·423	10·51

phate was injected in amount equivalent to 1·5 times the amounts of the salts used for the calcium injections. The rise in the serum-inorganic phosphorus was rapid and relatively prolonged: at the end of one hour it was 5·9 mg. (140 %) above the initial value, and at 2 hours was at a slightly higher level. At the same interval after the injection, the serum-calcium had fallen by 2·2 mg. or 17 %. Samples were unobtainable after 2 hours owing to the collapsed state of the animal.

From these results it is plain that the inverse relationship between the calcium and inorganic phosphorus of serum observed in the dietary experiments is only partially maintained when calcium or phosphate is injected. The results of the injection of phosphate are in accord with the dietary experiments inasmuch as the serum-calcium falls as the inorganic phosphorus rises. Several previous workers have reported a depression of the serum-calcium on injecting phosphate. Binger [1917] produced a fall of the serum-calcium to 6 mg./100 ml. with the development of tetany by injecting dogs intravenously with disodium phosphate. Tisdall [1922] repeated his experiments with a similar effect, while Salvesen *et al.* [1924, 1] obtained the same results when phosphates were administered orally. The calcium thus displaced from the serum is apparently largely excreted since Greenwald and Gross [1925] found a rise in the calcium excretion in dogs after the injection of neutral sodium phosphate, while Boyd *et al.* [1930] observed, in addition to a fall in the serum-calcium, an increased urinary excretion of calcium after injecting sodium glycerophosphate into the same animals.

In the present investigation injection of calcium has produced no definite fall in the serum-inorganic phosphorus, so that in this respect there is no effect analogous to the inverse relationship of the dietary experiments. The results of other workers are somewhat variable. Bomskov [1930] found that a fall in the

inorganic phosphorus followed the injection of calcium gluconate into rabbits. Salvesen *et al.* [1924, 2] and Collip [1926], using dogs, reported a small rise in the inorganic phosphorus value on injecting calcium chloride. Condorelli [1926] found a greater rise in the rabbit injected with calcium lactate. Greville [1931] found the inorganic phosphorus of the serum of two rabbits slightly elevated after the intravenous injection of calcium lævulate, while Apitzsch [1931] observed that the serum-inorganic phosphorus of human subjects was raised after the oral administration of calcium lactate. Thus the balance of evidence suggests that there is no disappearance of phosphorus from the blood, though it is noteworthy that Greenwald and Gross [1925] found an increased excretion of phosphorus in the dog after the injection of calcium chloride.

4. *The interrelations of magnesium and inorganic phosphorus.* Following the injection of magnesium (Table V) there is a fall in the serum-inorganic phosphorus amounting, after the injection of the sulphate to 0·9 mg. (32 %) and after that of the lactate to 0·5 mg. (15 %). The somewhat greater effect of magnesium sulphate is possibly accounted for by the synergic action of the sulphate ion which itself lowers the serum-inorganic phosphorus (Table VI). It will be seen that the injection of phosphate (Table VII) caused no appreciable change in the serum-magnesium, just as the injection of calcium caused no effect on the serum-inorganic phosphorus. These results are not in accord with the direct relationship between magnesium and inorganic phosphorus observed in the dietary experiments.

Discussion.

The injection experiments differ from the earlier dietary experiments in two important respects which may account to some extent for some of the divergences in the results of the two series. Firstly, whereas in the injection experiments salts were introduced subcutaneously and rapidly absorbed into the circulation, in the dietary experiments much smaller amounts of the relevant ion were slowly absorbed from the intestine over a period occupying 2 hours or more. Secondly, while two ions only were concerned in the injection experiments, a number of ions were being absorbed simultaneously in the dietary experiments, as well as other constituents which may themselves exercise important effects.

These differences do not appear to be of sufficient importance materially to affect the relationship existing between the calcium and magnesium of serum, which in both series of experiments is inverse. It is noteworthy that the effects of the injections have not been as drastic as might be anticipated. In the dietary experiments the rises and falls in the serum-calcium corresponded with similar rises and falls in the serum-magnesium, whereas in the injection experiments the greater and more rapid increase in the concentration of the one ion in the blood has produced a fall in the other hardly greater than those seen in strictly physiological conditions. It is evident that there exist mechanisms whereby the organism can resist attempts to lower a blood constituent to an undesirable level.

When the relations of calcium and magnesium to inorganic phosphorus are studied, differences in the results of the two series of experiments are at once apparent. Although the injection of phosphate causes a fall in the serum-calcium the converse is not observed, since when a calcium salt is injected, the serum-inorganic phosphorus is virtually unaffected. The inverse relationship between serum-calcium and inorganic phosphorus as observed in the dietary experiments therefore appears to operate in one direction only. The direct relationship between magnesium and inorganic phosphorus observed in the dietary experiments receives no support from the results of the injections, since while the

injection of phosphate leaves the serum-magnesium unaffected, the injection of magnesium salts depresses the inorganic phosphorus so that the two constituents are actually in inverse relationship.

The lack of reciprocity between calcium and inorganic phosphorus on the one hand and between inorganic phosphorus and magnesium on the other which is indicated by the results of the injection experiments appears to find an explanation when the relative concentrations of these constituents in the blood are considered. Normally the calcium of serum is more than equivalent to the inorganic phosphorus, so that it might be anticipated that, when more phosphate was introduced into the blood-stream, combination with calcium would occur with the deposition in some suitable site of calcium phosphate and a consequent fall in the serum-calcium value. Since calcium is present in excess of inorganic phosphorus, it is hardly surprising that the introduction of further calcium produces no fall in the inorganic phosphorus. Similarly, the inorganic phosphorus of serum is more than equivalent to the magnesium, so that, when more magnesium is introduced, it seems reasonable to expect that some of the magnesium will combine with inorganic phosphorus, the disappearance of which from the blood will lead to a fall in the serum-inorganic phosphorus. On the other hand, the addition of further phosphate when inorganic phosphorus is already in excess need hardly be expected to cause a fall in the serum-magnesium.

The injection experiments suggest that fluctuation of the serum-inorganic phosphorus is the chief controlling factor in the calcium-inorganic phosphorus relationship observed in the dietary experiments. This is in harmony with the explanation suggested by Fraser [1932] who regards the relationship as dependent on the periodical liberation of calcium phosphate from the bones. The calcium plays a passive rôle, in that calcium deficiency causes calcium phosphate to leave the depots with consequent rise in the serum-inorganic phosphorus value, whereas the presence of a sufficiency of calcium in the blood leads to a discontinuance of the process, so that the serum-inorganic phosphorus tends to fall. It is obvious that the depots must normally be replenished from time to time and it may fairly be assumed that a rise in the serum-inorganic phosphorus is instrumental in this effect leading to a deposition of calcium phosphate in the bones. Regarded from this aspect, the mechanism of the reciprocal relationship might be stated thus: A rise in the serum-inorganic phosphorus leads to a fall in the serum-calcium by deposition of calcium phosphate in bone, while a fall in the phosphorus value, as by excess of excretion over intake, allows liberation of calcium phosphate from the bones to proceed with consequent rise in the serum-calcium value.

Summary.

1. With a view to confirming earlier observations of the effects of the inorganic constituents of diet on the relationships between the concentrations of calcium, magnesium and inorganic phosphorus in the serum of the rabbit, solutions containing the appropriate ions have been injected subcutaneously and their effects on the three constituents compared with those noted previously.

2. The inverse relationship existing between the concentrations of calcium and magnesium in the serum, first observed when rabbits are kept on certain diets, has been confirmed by the subcutaneous injection of calcium and magnesium salts.

3. The inverse relationship between the serum-calcium and inorganic phosphorus and the direct relationship between the serum-magnesium and inorganic phosphorus is only supported in so far as the injection of disodium phosphate

a fall in the serum-calcium. The injection of the gluconate, laevulate and ...te of calcium has no constant effect on the serum-inorganic phosphorus, nor ...the injection of phosphate affect the serum-magnesium. The injection of the ...ate and lactate of magnesium depresses the serum-inorganic phosphorus, ...the relationship is therefore the converse of that observed in the dietary ...ments.

4. The above findings are discussed in relation to the concentration of ...um, magnesium and inorganic phosphorus, normally existing in the blood.

5. The injection of sodium sulphate depresses the serum-calcium and in... phosphorus but leaves the serum-magnesium unchanged.

6. The injection of sodium chloride has no significant effect on the serum-...um, magnesium or inorganic phosphorus.

7. Small haemorrhages cause a slight temporary depression of the serum-...um.

The writer wishes to record his thanks to the British Medical Association for ...ward of a Research Scholarship, during the tenure of which the work was ...ly carried out.

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Comparison of Parenterally Administered Calcium Kinate Gluconate with Calcium Gluconate and Calcium Chloride

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The homeostatic nature of the human body requires that there be a continuous intake of calcium. A sufficient amount of calcium is obtained from a normal diet, and the dietary sources of calcium need to be supplemented by extra dietary administration only in abnormal circumstances. The use of calcium therapy in clinical conditions is not always associated with actual deficiency states. Evidence has been presented in the past that parenterally administered calcium aids in the control of edema (Morris and Rogen, 1940; Lecomte, 1950), allergy (Rudolph, 1936; Sullivan, 1941), urticaria (Chambers and Bernton, 1944; Parker, 1950), lead poisoning (Shields and Mitchell, 1941), and intestinal, renal, and biliary colic (Bauer *et al.*, 1931).

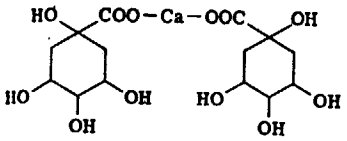
Excessively large quantities of calcium salts have been cited as possessing an anticoagulant effect (Loomis and Seegars, 1944). The addition of small quantities of calcium to normal blood is generally not considered to have a significant effect.

The studies reported here are concerned with the experimental evaluation of the toxicity of calcium kinate gluconate in comparison with that of calcium gluconate and calcium chloride.

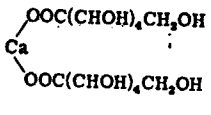
MATERIALS AND METHODS

Calcium kinate gluconate (CKG) prepared by Albro and Buck (1957) is a highly soluble complex of calcium kinate and calcium gluconate in an approximate molar ratio of 2:1. The complex yields a higher concentration of calcium than is obtainable from saturated solutions of either

component alone. The calcium kinate gluconate solution used in this experiment was water clear and contained 50 mg of elemental calcium per milliliter.



Calcium kinate



Calcium gluconate

Calcium gluconate (CG), 10% solution, USP (E. R. Squibb and Sons and the Upjohn Co.), the generally accepted parenteral formulation of calcium, and calcium chloride ($CaCl_2$) were used as standards for comparison in most instances during these studies. The various experimental procedures are described in detail in this section.

Acute Intravenous Toxicity

Mice in groups of ten were injected intravenously with calcium kinate gluconate (CKG), calcium gluconate (CG), or calcium chloride ($CaCl_2$). The LD_{50} was calculated according to the logarithm dose-probit method of Miller and Tainter (1944).

Acute Intravenous Tolerance

Two groups of six unanesthetized dogs were injected intravenously with calcium kinate gluconate or calcium gluconate. One week later a crossover experiment was done in which each dog was injected with the alternate solution. The preparations were injected into the saphenous vein at a rate of 3 mg Ca per kilogram per minute. This rate of injection was approximately twice that which is recommended for therapeutic purposes. Maximum calcium tolerance was determined at the moment vomiting occurred and the total milligrams of calcium of the injected solution per kilogram body weight was noted.

Antagonism to Magnesium Toxicity[1]

The comparative availability of calcium ion in calcium kinate gluconate, calcium gluconate, and calcium chloride preparations was determined by the antagonism of calcium ion to the toxicity of magnesium ion. Solutions

[1] The authors wish to thank Dr. James O. Hoppe for this study.

of the three calcium preparations with a constant 5:6 ratio of calcium ions to magnesium ions (as magnesium sulfate) were injected intravenously into mice. The injected quantity of magnesium sulfate was sufficient to kill ten out of ten mice. Survival of the mice injected with an antidotal dose of calcium was used as a measure of the availability of calcium ions.

Cardiovascular Effects[2]

The effects of calcium kinate gluconate on the heart and on the blood pressure were evaluated in dogs in comparison with calcium gluconate. Four dogs were anesthetized intravenously with approximately 15 mg/kg of sodium thiopental followed by 250 g/kg of sodium barbital. Femoral blood pressure was recorded by means of a P23A pressure transducer (Statham) supplying a Grass balance demodulator unit and an encephalograph. Lead I and II electrocardiograms were obtained simultaneously with the above recordings. CKG or CG were injected intravenously in doses ranging from 1.35 mg Ca to 7.5 mg Ca per kilogram at a rate of 7.5 mg Ca per kilogram per minute. The electrocardiograms and blood pressures were recorded prior to the injections and at 10, 20, 30, 60, 90, 120, 180, and 240 minutes after the injections. A crossover experiment was done on the same day so that each dog received both solutions.

Intramuscular Tolerance

This study was done to determine the irritation of calcium kinate gluconate when injected intramuscularly at various concentrations in the rabbit. Calcium gluconate was injected and studied as a standard preparation. Twelve adult albino rabbits were divided into six groups of two rabbits each and were injected as shown in the tabulation.

Group	Drug	Ml injected	Mg Ca injected
I	CKG	1.0	5.0
II	CKG	1.0	10.0
III	CKG	1.0	15.0
IV	CKG	1.0	25.0
V	CKG	1.0	50.0
VI	CG	1.0	10.0

Each rabbit was injected with the test solution into three different muscles on the first, sixth, and seventh days. On the eighth day, the

[2] The authors are thankful to Dr. Leonard Grumbach for this study.

rabbits were sacrificed with intravenous sodium thiopental and the injection sites of 1, 2, and 7 days' duration were examined grossly. The tissues of the injection sites were fixed in Zenker-formalin, stained by the hematoxylin and eosin method, and examined microscopically.

Chronic Intravenous Tolerance

Fifteen dogs were divided into five groups of three dogs each. Calcium kinate gluconate was administered intravenously to two groups at 2 or 3 mg Ca per kilogram, calcium gluconate to one group at 10 mg Ca per kilogram, and sodium kinate to two groups at 75 or 375 mg/kg. A sixth group of two dogs received physiological saline as a control of the experimental procedure. Sodium kinate was administered as a control of the effects of kinic acid, one of the constituents of the calcium kinate gluconate complex. All preparations were injected three times weekly for 6 months.

The dogs were observed carefully, during and after medications, for changes in general appearance and behavior. Body weights and rectal temperatures were determined weekly. Catheterized urine specimens were examined three times during the experiment to determine the effects of the drugs on the color, odor, turbidity, and the chemical and microscopic properties of the urine. At the end of the experiment, the control and medicated dogs were sacrificed with intravenous sodium thiopental and the thoracic and abdominal viscera were examined grossly. Sections of tissues were fixed in Zenker-formalin, stained by the hematoxylin and eosin method, and examined microscopically.

Hematologic Studies

Hematologic studies were done on the same 17 dogs used for the chronic 6-month intravenous tolerance study. The dosage regimen of these dogs is described in the previous section.

Another experiment was done with monkeys. Six rhesus monkeys (*Macaca mulatta*) were divided into three equal groups. CKG or CG at 3.5 mg Ca/kg or saline in an equivalent volume to the CG dosage were injected three times intravenously on alternate days to one group each. Total red and white blood cell counts, differential counts, hematocrits, and hemoglobin concentrations were determined before medication and approximately once a month thereafter on dogs, and before medication and at 1 and 4 days after the last injection on monkeys. Platelet counts were made, by the Rees-Eckert method, from the blood of dogs twice

during the fourth and fifth months, and of monkeys following injection. Clotting time of blood from dogs receiving the high calcium and from the control dogs was determined by the capillary tube method three times during the fourth and fifth months; clotting time of blood from monkeys was determined after each injection. Serum calcium levels were determined at monthly intervals on dogs by the Cramer-Tisdall method, and following each injection on monkeys by the colorimeter method of Kibrick *et al.* (1951).

RESULTS

Acute Intravenous Toxicity

Calcium kinate gluconate and calcium gluconate had approximately the same acute intravenous toxicity in terms of 24-hour LD_{50} and on the basis of total compound administered, but they were less toxic than calcium chloride. On basis of calcium content, no significant difference in the acute intravenous toxicity was observed among the three compounds. The 7-day LD_{50} was the same as that at 24 hours. These data are presented in Table 1.

TABLE 1

ACUTE TOXICITY IN MICE INJECTED INTRAVENOUSLY WITH CALCIUM SALTS

Medication	I.v. 24-hr[a] LD_{50} (mg/kg ± S.E.)	
	As Compound	As Ca
Calcium kinate gluconate	1050 ± 57	83 ± 4
Calcium gluconate	950 ± 83	86 ± 8
Calcium chloride	215 ± 14	78 ± 5

[a] Same LD_{50} at 7 days.

Acute Intravenous Tolerance

Calcium kinate gluconate was better tolerated than calcium gluconate in terms of total Ca administered when injected intravenously into dogs. The mean maximum tolerated dose of CKG as determined by occurrence of vomiting was 19.7 ± 2.6 mg Ca per kilogram and that of CG was 13.2 ± 1.7 mg Ca per kilogram.

Antagonism to Magnesium Toxicity

Injections of magnesium sulfate ($MgSO_4$) alone at a dosage of 48 mg/kg produced death in all mice. The death rate was reduced sharply when one of the three calcium salts was added at 40 mg Ca per kilogram to the injections of $MgSO_4$. No definite difference in effectiveness was

observed among the three calcium preparations. As a control, a volume of saline equal to that of the calcium solution was added to the $MgSO_4$ solution; no decrease in toxicity was observed, a result indicating that the marked reduction in mortality of the magnesium was not due to dilution of the magnesium solution by the calcium solution: this reduction was caused by the addition of the calcium salts. The results of this experiment are presented in Table 2.

TABLE 2

ANTAGONISM OF CALCIUM SALTS TO MAGNESIUM TOXICITY IN MICE

Medication	Dose mg/kg i.v. as Ca	Dose mg/kg i.v. as Mg	Mortality No. dead/no. injected
Calcium kinate gluconate	40	—	1 / 10
Calcium gluconate	40	—	1 / 10
Calcium chloride	40	—	1 / 10
Magnesium sulfate ($MgSO_4$)	—	48	20 / 20
Calcium kinate gluconate + $MgSO_4$	40	48	2 / 20
Calcium gluconate + $MgSO_4$	40	48	3 / 20
Calcium chloride + $MgSO_4$	40	48	2 / 20
Saline + $MgSO_4$	—	—	10 / 10

Cardiovascular Effects

Calcium kinate gluconate or calcium kinate produced no significant changes in the electrocardiograms, heart rates, or blood pressures in dogs when injected intravenously at doses up to 7.5 mg Ca per kilogram.

Intramuscular Tolerance

There was no apparent evidence of pain in any of the rabbits injected intramuscularly with calcium kinate gluconate or with calcium gluconate. The skin at the sites of injections was normal in appearance. The general gross and microscopic observations differed primarily in the severity of the lesions produced by the calcium preparation rather than in the type of inflammation.

CKG was well tolerated when injected at 5 or 10 mg Ca per dose, as was CG at 10 mg Ca per dose. Minimal tissue damage attributable to the medication at these dosages was observed at 24 and 48 hours, and healing was well advanced by the seventh day.

Intramuscular injections of CKG at 15 or 25 mg Ca per dose were well tolerated, but moderate tissue damage was observed at 24 and 48 hours. Although the nature of the tissue reaction was similar following these

two doses, the 25 mg Ca per dose resulted in larger areas of inflammato reaction. Healing was advanced by the seventh day.

Intramuscular injection of CKG at 50 mg Ca per dose was not as w tolerated as the more dilute solutions. Moderate to severe tissue dam was observed at 24 and 48 hours and the rate of healing was slower.

Chronic Intravenous Tolerance

The dogs injected intravenously with calcium kinate gluconate calcium gluconate three times weekly for 6 months were normal in appea ance and behavior throughout the experiment. The body weight gain the medicated and control dogs was normal. No symptoms of hyp calcemia occurred and no evidence of irritation was observed in any the sites of repeated intravenous injections at any time. The rectal te temperature of the medicated dogs was within normal limits through the experiment.

No significant change attributable to medication occurred at any ti in color, odor, turbidity, or the chemical or microscopic properties urine of the dogs. No significant macroscopic or microscopic lesi attributable to medication were observed in any of the dogs autops at the termination of the experiment.

Hematologic Studies

Total red and white blood cell counts, differential counts, hematocrit and the hemoglobin concentrations of dogs or monkeys injected wi calcium kinate gluconate or calcium kinate were normal as compared control dogs or monkeys.

Clotting time in *dogs* injected with the calcium solutions at 10 mg per kilogram or with sodium kinate at 375 mg/kg for 3–4 months w faster than in the controls. Usually, this was observed within 15 minut after each injection, and the clotting time returned to the premedicati time within 45 minutes. Determinations made at 60 minutes and at and 2 days following the last injection were within normal limits. A d crease in clotting time was noted also in both groups of *monkeys* me cated with CKG or CG. The decrease was observed within 15 minu after each injection and reached a maximum by 30 minutes. The clotti time returned to normal at 90 minutes following injections of C whereas after CKG the clotting time returned to normal at 150 minu or more. Four days after the last injection the clotting time of monkeys was normal. The clotting time values are presented in Table

TABLE 3

CLOTTING TIME IN DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication[c]	Species	Clotting time								
			Time after medication							
		Pre-med.	5′	15′	30′	60′	90′	150′	24 Hr	48 Hr
Saline control	Dog[a]	2′15″	—	2′57″	2′30″	2′57″	—	—	2′30″	2′50″
CKG 10 mg Ca/kg	Dog	2′55″	—	2′45″	2′01″	2′53″	—	—	2′48″	2′57″
CK 10 mg Ca/kg	Dog	2′20″	—	1′51″	1′40″	2′18″	—	—	2′27″	2′27″
Na kinate 375 mg/kg	Dog	2′30″	—	2′21″	1′53″	2′41″	—	—	2′43″	2′48″
Saline control	Monkey[b]	1′23″	1′18″	1′18″	1′18″	—	1′18″	—	—	—
CKG 7.5 mg Ca/kg	Monkey	1′27″	1′18″	0′51″	0′41″	—	0′53″	1′08″	—	—
CG 7.5 mg Ca/kg	Monkey	1′31″	1′15″	0′53″	0′41″	—	1′23″	—	—	—

[a] Averages of 3 dogs in each medicated group and 2 in the controls at 3–4 months of medication.
[b] Averages of 2 monkeys in each group following 3 injections on alternate days.
[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

A slight increase in platelet counts was observed in dogs and monkeys injected with CKG and in dogs injected with sodium kinate within 15 or 30 minutes after the injections. No definite increase was noted in dogs or monkeys injected with CG. The platelet counts are presented in Table 4.

TABLE 4
PLATELET COUNTS OF DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication[c]	Species	Platelet count (1000/mm³)						
		Pre-med.	Time after medication 5′	15′	30′	60′	90′	24 H
Saline control	Dog[a]	400	—	—	354	454	—	[illegible]
CKG 10 mg Ca/kg	Dog	389	—	—	475	476	—	[illegible]
CK 10 mg Ca/kg	Dog	422	—	—	435	380	—	[illegible]
Na kinate 375 mg/kg	Dog	379	—	—	435	448	—	[illegible]
Saline control	Monkey[b]	296	287	301	300	—	305	
CKG 7.5 mg Ca/kg	Monkey	292	361	407	429	—	375	
CG 7.5 mg Ca/kg	Monkey	412	407	445	405	—	387	

[a] Averages of 3 dogs in each medicated group and 2 in the controls at [illegible] months of medication.

[b] Averages of 2 monkeys in each group following three injections on alternate days.

[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

A significant increase in the serum calcium level was observed in the dogs injected with CKG or CG when compared to the controls. The maximum increase was reached within 15 minutes following the injection and was still slightly elevated 3 hours later. Similarly, a significant increase was observed in the monkeys injected with CKG or CG when compared to the controls. This increase reached a maximum within minutes following the injections and returned to an approximately normal level within 90 minutes. Serum calcium levels are presented in Table

Clinical Studies

Detailed clinical reports (Nobel, 1959) on over 200 patients treated with calcium kinate gluconate have been analyzed. These reports indicated excellent tolerance of CKG. Besides the transient flushing or feeling of warmth experienced by many patients receiving parenteral calcium, the only reported untoward reaction was a temporary dizziness felt by two asthmatic patients receiving an intravenous solution containing dexte-

Benadryl, ACTH, and CKG. The number of treated patients included 125 cases of dermatoses, 18 cases of asthma, 4 cases of lead poisoning, and 60 cases in a state of calcium deficiency.

TABLE 5
SERUM CALCIUM VALUES AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication[c]	Species	Serum calcium (mg/100 ml) at minutes after injection							
		Pre-med.	5′	15′	30′	60′	90′	120′	180′
Saline control	Dog[a]	10.3	—	10.4	—	10.5	—	10.7	10.7
CKG 10 mg Ca/kg	Dog	10.3	—	14.4	—	13.2	—	12.7	12.0
CG 10 mg Ca/kg	Dog	10.7	—	16.1	—	14.0	—	13.4	12.2
Na kinate 375 mg/kg	Dog	10.7	—	10.9	—	10.5	—	10.7	10.5
Saline control	Monkey[b]	11.8	11.3	11.5	11.6	—	11.8	—	—
CKG 7.5 mg Ca/kg	Monkey	10.8	14.2	13.5	13.0	—	11.7	—	—
CG 7.5 mg Ca/kg	Monkey	12.1	15.0	14.3	13.4	—	12.5	—	—

[a] Averages of 3 dogs in each medicated group and 2 in the controls from 6 determinations at monthly intervals following chronic administration.

[b] Averages of 2 monkeys in each group following three injections on alternate days.

[c] CKG = calcium kinate gluconate; CG = calcium gluconate.

DISCUSSION

The pharmacologic activity of calcium kinate gluconate was similar to that of calcium gluconate and was apparently due to the calcium ion. No significant difference was observed among CKG, CG, or $CaCl_2$ in protecting mice against a lethal dose of magnesium when the doses were calculated in terms of their calcium ion content. The acute intravenous toxicity of these compounds also appeared to be directly related to the calcium ion when calculated in terms of the calcium content of these preparations. On a molecular basis, CKG was the least toxic of the three compounds. No chronic toxicity was produced by either CKG or CG.

The degrees of intramuscular irritation were similar for CKG and CG when administered at an equal calcium concentration. If the CKG concentration was decreased, the degree of intramuscular irritation decreased proportionally. CKG was well tolerated with calcium concentrations up to 25 mg/ml.

The specific effect of the calcium ions was less evident in acute intravenous tolerance studies in dogs where CKG was better tolerated than

CG when administered under identical conditions and at the same rate of calcium injection. It is possible that the anionic moiety of CKG increases the tolerance of the recipient to calcium.

Another possible difference between CKG and CG was observed in hematologic studies where slightly increased platelet counts were noted in dogs and monkeys following injections of CKG but not of CG. However, both Ca preparations shortened blood clotting time. These effects of CKG and CG could not be attributed only to high serum calcium levels since injections with sodium kinate into dogs also resulted in a shorter blood clotting time with a possible slight increase in platelet counts. A similar observation with kinic acid has been reported by Noda and Kurakake (1939): kinic acid injected intravenously into rabbits reduced blood coagulation time. The effect of CKG on blood clotting time and platelet counts may be attributable to the presence of Ca ions and kinic acid.

CKG is of interest in the treatment of lead poisoning. In recent years chelating agents have been successfully used to tie up heavy metals. CKG in these cases may be used similarly in view of its chelating properties of the kinic acid moiety, superimposed on the mobilizing effect of calcium.

SUMMARY

The pharmacologic and toxic properties of calcium kinate gluconate, calcium gluconate, and calcium chloride have been studied in mice, dogs, and monkeys. The pharmacologic properties appeared to be based on the calcium concentration except for acute intravenous tolerance where calcium kinate gluconate was better tolerated than calcium gluconate. Calcium kinate gluconate and calcium gluconate injections produced transient decrease in blood clotting time, but increased platelet counts were observed only following calcium kinate gluconate injections. The calcium kinate gluconate effect on blood probably was attributable to the presence of calcium and kinic acid. No toxic properties of calcium kinate gluconate and calcium gluconate were observed in a 6-month tolerance study in dogs. Good clinical tolerance of calcium kinate gluconate was reported on over 200 cases.

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Effects of the Intravenous Injection of Certain Salts of Sodium, Calcium and Potassium on Intestinal Tonus and Motility in the Dog*

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For MANY YEARS saline purges have been administered by the mouth but, recently, certain salts also have been injected intravenously to stimulate the intestinal tract.

This study was undertaken with the object of determining the ability of certain sodium, potassium and calcium salts to stimulate the muscular activity of the intestine in the dog.

Two types of experimental procedure were followed. Type 1 required an anesthetized dog and involved a recording of the respiratory movements, of the blood pressure from the carotid artery and of the intestinal movements as revealed by a balloon placed within the small intestine. Type 2 provided for a recording of respiratory movements and a recording of intestinal movements as revealed by a jacket placed about an exteriorized skin-covered loop of small intestine.

Experimental Procedure

In type 1, the dogs were anesthetized with pentobarbital sodium dissolved in distilled water and administered by intrapleural injection at the rate of 30 mg. per kilo (2.2 lbs.) of body weight. A 6 per cent solution was used.

The records were made on long smoked paper revolving on a Miller kymograph. Blood pressure was recorded by a mercury manometer connected by means of a three-way cannula with the left carotid artery. The cannula also was connected with a pressure bottle containing an anticoagulant (8 per cent sodium citrate solution).

Respirations were recorded by means of a pneumograph connected with a Becker air tambour. Movements of the intestine were recorded by the balloon method. By means of a median incision the anterior portion of the jejunum was isolated and exposed. A thin rubber balloon attached to a human catheter was inserted into the lumen through a small incision in the intestinal wall. The incision in the wall of the intestine was closed by a purse-string suture which was drawn tightly around the catheter connected to the balloon. The intestine was replaced in the abdominal cavity and the abdominal incision was closed by clamping with hemostats. The catheter was attached by tubing to a burette partly filled with water; the water was allowed to flow into the balloon, thus distending it. The top of the burette was connected by rubber tubing with a Macey air tambour. The movements of the intestine exerted a pressure on the water in the balloon that was transmitted to the column of water in the burette. The column of water compressed the air in the top of the burette and transmitted the variation to the recording tambour.

A burette for injection purposes was connected by means of rubber tubing to a three-way cannula inserted into the femoral vein. A three-way cannula was used so that it could be washed out between injections, if necessary. Special pipettes to control the rate of injections were inserted in the tubing connecting the burette with the cannula. The pipette most frequently used allowed 100 cc. (3.3 oz.) of the solution to pass in eight minutes.

In type 2, a method that is new in the study of intestinal motility is based on an operation on the dog first performed by Biebl[1] and improved by Bors and Polano.[2] The method used in these experiments was adapted largely from the technic of Bors

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and Polano. Two parallel skin incisions, about 6 in. in length, were made longitudinally about 1½ to 2 in. apart in the loose skin of the right flank. The subcutaneous fasciae were torn loose underneath the strip of skin on all sides. A 3- or 4-in. incision through the abdominal muscles and peritoneum was made underneath the median skin wound. The first part of the jejunum was carefully located and brought through the opening in the abdominal wall. A section of mesentery was selected that was comparatively free of blood vessels and nerves. The section was cut away from the intestine and care was taken not to injure neighboring vessels or nerves. The strip of skin was folded around the free loop of intestine and the edges fastened together with interrupted silk sutures. The peritoneum above was sutured with catgut; then the abdominal muscles in like manner. The free skin lateral to each of the parallel incisions was stretched until both parts met underneath the loop and were sutured with silk.

The wounds were protected by a double layer of sterile gauze cut in two sections to fit the area and held in place by special adhesive cement. The bandage was examined on the second day following the operation and loosened if it was too tight; it was removed on the third or fourth day. The silk sutures usually were removed a few days later. A broad fiber-board collar was used to keep the dog from annoying the wound.

In 1½ to two weeks following the operation the wound was sufficiently healed so that it could be measured and a metal jacket was made to fit the intestinal loop of the individual dog. The jacket was made of a hollow, metal cylinder that was cut down the middle from top to bottom and hinged so that it would open to permit the entrance of the intestinal loop. The cylinder was about one-fourth inch larger on all sides than the skin-covered intestine. At both ends of the cylinder a metal collar one-fourth inch wide was soldered so as to fit moderately close about the intestine and to close the end of the cylinder. A metal tube about one inch in length and one-half inch in diameter was soldered to an opening in the middle of the cylinder. A rubber balloon was attached to a three-eighths inch rubber tubing by a rubber band and the balloon was cemented inside the metal jacket. The rubber tubing was put through the short metal tube leading from the side of the jacket and attached to a water-recording meter with a writing point. The rubber balloon and tubing were carefully completely of all air and filled with water. A water pressure of about 15 cc. (0.5 oz.) was exerted on the balloon.

While the wound was healing, each dog was trained to lie quietly on the table. The dogs maintained their positions for periods sometimes as long as 12 hours without appreciable movement. Some of them permitted repeated intravenous injections into the cephalic vein without interference with the recordings. After the dogs became accustomed to the procedure they usually slept at intervals during the experiment.

Records of respiration were made at the same time that the intestinal movements were being recorded. The purpose of the respiratory tracing was not to study respiration only but to note the possible influence of respiration upon the intestinal movements. The respiratory movements were usually altered when the animal moved, which helped to explain unusual movements of the intestine.

A minimum of three days was allowed between the periods when an individual dog was used for different or repeated experiments. Thus, sufficient time was considered to have elapsed for the excretion of salts previously injected.

All of the experiments in type 2 were performed on unanesthetized dogs. An outstanding advantage of the method was that intestinal motility was not influenced by the anesthetic. Another advantage was the possibility of using the same dog for several experiments and studying the effects of different substances so that a comparison of reactions in the same animal could be made.

Discussion

Two terms are used here to describe intestinal activity. The term intestinal motility or movement is used to refer to the

by varying segmentations and peristaltic waves; the term intestinal tonus is used to refer to the slowly changing state of generalized contraction.

Movements of the intestine, such as localized segmentation, peristalsis and antiperistalsis, could not be identified individually by the methods used to record intestinal activity. Therefore, the general terms, motility or movement, were used to indicate segmentation or peristalsis of the intestine. Variations in intestinal tonus were easily recognized.

Lang's Solution.—Lang's solution was an effective agent in stimulating both intestinal tonus and motility. The first response was an increase in motility which was followed immediately by an increase in intestinal tonus. Intestinal motility appeared to be more easily stimulated than intestinal tone.

In the anesthetized animal Lang's solution seemed to exert its most pronounced action on the respiratory system. The smallest injection caused rapid and shallow breathing. Each injection produced a marked fall in blood pressure, although the heart continued to beat strongly.

In the unanesthetized dogs Lang's solution did not produce undesirable reactions upon the respiratory or circulatory systems. However, much smaller amounts of solution were administered in these cases than were given to the anesthetized dogs.

In none of the dogs, either anesthetized or unanesthetized, did the induced intestinal activity produce defecation. In general, Lang's solution produced strong intestinal activity although in some cases rather long intervals elapsed between the administration of the Lang's solution and the reaction.

Lang's solution consists of 60 Gm. (2 oz.) each of sodium chloride and sodium citrate dissolved in one quart of water.

Sodium Chloride.—Solutions of sodium chloride varying in strength from 2.5 per cent to 12.5 per cent were used. Each of the seven experiments with sodium chloride gave approximately the same results. Intestinal motility and tonus were stimulated by each of the different strengths administered. The use of different concentrations produced responses varying only in proportion to the amount of salt injected. The total amount of salt rather than the percentage strength of the solution injected appeared to be the factor influencing intestinal activity.

An early effect, more noticeable following the administration of the dilute rather than the stronger solutions of sodium chloride, was the stimulation of intestinal contractions without an increase in tonus. After additional saline had been injected, intestinal tonus was stimulated as strongly as intestinal contractions. Large amounts of saline produced extreme increases of intestinal tonus accompanied by suppression of motility. It appeared that the movements of the intestine, such as rhythmic segmentations and peristaltic rushes, possessed a lower threshold value than did the mechanism for increasing intestinal tonus.

Intravenous injections of solutions of sodium chloride were relatively nontoxic. Neither the circulatory nor respiratory systems were particularly susceptible to sodium chloride even though all the solutions were hypertonic. Hewitt, Greenwood and Nelson[7] showed that 60.6 Gm. (2 oz.) of sodium chloride in 30 per cent solution injected intravenously over a period of 17 minutes proved fatal to a dog weighing 18.18 kg. (40 lbs.). This was equivalent to 3.3 Gm. (.11 oz.) per kilogram of body weight. They concluded that the toxicity of sodium chloride was effected in three ways: 1) The osmotic effect, which increased blood volume and hence blood pressure; 2) a direct effect upon the medullary centers, at first stimulating and later paralyzing these centers; and 3) increasing the permeability of the cell membranes, causing fluid to escape more readily into the tissue spaces.

Sodium Citrate.—The response of the intestine to injections of sodium citrate was the opposite to that caused by sodium chloride. The citrate produced an increase of intestinal tonus with smaller amounts of solution than were needed to stimulate intestinal movements. It would appear that tonus was more susceptible to injections of sodium citrate than was the mechanism initiating intestinal movements. Intravenous injections of sodium citrate were relatively

toxic to both the circulatory and the respiratory systems.

With the intention of noting possible differences in the stimulating actions of the citrate ion and the chloride ion, solutions of citric acid and hydrogen chloride gas were injected intravenously into different anesthetized dogs. The results of administering a solution of hydrogen chloride gas to 4,500 indicated that the chloride ion did not stimulate intestinal activity. Similarly, a 5 per cent solution of citric acid was injected into each of three anesthetized dogs and in no case was intestinal motility or tonus stimulated.

By a process of elimination the citrate and the chloride ions did not seem to be the factors concerned in stimulating intestinal activity. Only the sodium ion remained to account for the stimulation of the intestine produced by sodium chloride and sodium citrate. The manner in which the sodium ions exerted their influence was not definitely revealed. Hammett[1] and Hammett and Nowrey[5] suggested that the sodium ion might act by increasing the permeability of the tissue to some other agent initiating the response. Hughson and Scarff[8] concluded that the sodium chloride had a direct effect upon the muscle fibers of the intestinal wall.

Sodium Bicarbonate.—A 5 per cent solution of sodium bicarbonate stimulated the small intestine of the dog more effectively than any of the solutions injected. Strong movements of the intestine were continued longer under the influence of sodium bicarbonate than any other salt. Comparatively large injections of sodium bicarbonate stimulated intestinal tonus so intensely that localized movements of the intestine were inhibited.

No reaction was elicited from the respiratory or circulatory systems by intravenous injections of moderate amounts of sodium bicarbonate. Over 600 cc. (20 oz.) of a 5 per cent solution was injected into one animal without producing an outstanding reaction.

Calcium Chloride.—Calcium chloride in 1 per cent solution did not stimulate intestinal activity as much as did the sodium salts. However, the influence of the injection seemed to persist longer with calcium chloride than with the sodium salts.

There has been considerable difference of opinion regarding the effect of calcium chloride upon intestinal movements.

Calcium Gluconate.—The intravenous injection a 5 per cent solution of calcium gluconate stimulated the small intestine more than did calcium chloride. Calcium gluconate did not affect the respiratory or circulatory system to any noticeable extent.

Calcium gluconate is soluble to the extent of about 3 per cent in 100 cc. (3.3 oz.) of distilled water, but by the addition of boric acid the solubility can be increased to 20 and even 30 per cent. The solution used in the experiments with calcium gluconate was stabilized with 4 per cent boric acid. In order to determine the effect of boric acid on intestinal motility, a 4 per cent solution of boric acid was administered to an anesthetized dog. Neither intestinal motility nor tonus was influenced by the injections. The respiratory and circulatory systems were not significantly altered by the injections. Thus, the results obtained from injecting calcium gluconate suspended in a solution by the aid of boric acid were considered to be due to the calcium salt and not to the boric acid.

Potassium Bicarbonate and Potassium Chloride.—Intestinal activity was inhibited in the experiments involving the intravenous injection of 5 per cent solutions of potassium bicarbonate and potassium chloride. These results were in agreement with Hazard and Wurmser[6] and Melnikov.[10] On the other hand, In[9] and Constantini and Ballarin[3] found that potassium chloride given intravenously stimulated intestinal movements. Considerable difference of opinion is evidenced in the literature in regard to the effect of potassium chloride on the intestine.

SUMMARY

The technic employed in experiments of type 1 permitted a study of the effects of intravenous injections upon the respiratory and circulatory systems as well as the intestinal musculature. This technic allowed a careful study of variations in intestinal

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Experiments of type 2 were performed on unanesthetized dogs that led a normal existence before, during and after the experiment. In this method the variations in blood pressure were not studied and the toxicity of an injected substance could be measured only by ordinary clinical methods.

By employing both types of procedure and comparing the results of each method, relatively inclusive data should be accumulated because the disadvantages of one method were nullified by the advantages of the other method.

A total of 30 experiments were performed. In type 1, 21 dogs were anesthetized and subjected to the experimental procedure. In type 2, nine experiments were performed with five unanesthetized dogs.

CONCLUSIONS

Lang's solution is an effective agent in stimulating both intestinal tonus and motility. However, it is slightly toxic to the respiratory and circulatory systems of the anesthetized dogs.

Solutions of sodium chloride stimulate the intestinal musculature. The first injections of small amounts stimulate intestinal motility primarily. Subsequent injections of larger amounts of sodium chloride solution stimulate intestinal tonus but suppress motility somewhat.

The quantity of sodium chloride injected rather than the percentage strength of the solution appears to be the factor stimulating the intestinal musculature. The response of the intestine varies in proportion to the amount of salt injected.

Large amounts of hypertonic solutions of sodium chloride administered intravenously are relatively nontoxic to the circulatory and respiratory systems.

An increase in tonus was the first and most persistent response of the intestine to the intravenous injection of sodium citrate. Intestinal motility is stimulated moderately by the citrate. A bradycardia and slowing of respiration result from large injections of sodium citrate.

The sodium ion appears to be instrumental in producing a stimulation of intestinal musculature. Sodium chloride and sodium citrate both stimulate the intestine but citric acid and a solution of hydrogen chloride gas do not affect the intestine. Therefore, it appears that the citrate and chloride ions do not stimulate the intestine. The sodium ion appears to be the only remaining factor and seems to be responsible for the stimulation of the intestinal musculature. The method whereby the sodium ion may be effective is not understood.

Lang's solution appears to stimulate the intestinal musculature by virtue of the sodium ions present.

Sodium bicarbonate stimulated the intestinal tonus and motility more effectively than any of the solutions used. The bicarbonate produced little or no effect on the respiratory and circulatory systems.

Calcium chloride did not stimulate the intestine as much as did the sodium salts but the effects persisted longer. Considerable danger of heart block accompanies the intravenous injection of calcium chloride.

Calcium gluconate stimulated the intestinal musculature more than calcium chloride. Boric acid, which was used to increase the solubility of calcium gluconate, apparently had no effect on the intestine.

The respiratory and circulatory systems were not altered significantly by the intravenous injection of calcium gluconate.

Potassium chloride and potassium bicarbonate appeared to depress the intestinal musculature. Potassium chloride was exceedingly toxic to the heart.

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Heartworms

A study has been made on the survival and location of the microfilariae of *Dirofilaria immitis* in the dog.

An uninfected dog was injected intravenously with blood containing approximately 233,000 microfilariae. They survived in the blood stream of this dog for more than two years. None could be found, however, upon necropsy 2½ years after injection. No increase in the size of the larvae was noted. Comparatively few of the larvae appeared in the peripheral circulation after injection. Probably a part of the microfilariae are concentrated in the capillary networks throughout the body, and a part leave the blood stream altogether. There is some evidence that the macrophage system, activated by some type of immunological reaction, may destroy large numbers of microfilariae in a short time, but such reactions are of irregular occurrence and they do not regularly affect microfilarial longevity or periodicity. (*P. C. Underwood and P. D. Harwood. Survival and Location of the Microfilariae of Dirofilaria Immitis in the Dog. Journal of Parasitology, xxv, 1939, pp. 23-33.*)

Exanthematous Typhus in Cats

When fed upon or inoculated with typhus-infected material taken from guinea pigs, cats are capable of having an inapparent form of exanthematous typhus. The disease thus produced is not febrile, but the virus can be put in evidence in the brain of the cat 37 hours after the inoculation, but, however, to lose its pathogenicity after 42 days. The authors showed that three cats which had been in contact with human cases contracted the disease in inapparent form. (*Abst., Revue de Médecine Vétérinaire, xci, April 1939, p. 221.*)

Mustard Gas

The veterinary corps of nations at war need not be reminded of the terrors of mustard gas for man and animals. Mustard gas is thiodiglycol chloride. Its pet name in World War I was "Yperite," named for Ypres, where it was first used. The date was July 12, 1917. It differs from the other war gases in having a destructive external action in addition to its irritant effect on the respiratory tract. No systemic action has been observed. It disables and kills by its topical action. The fluid or its vapor in horses destroys the epithelial and subepithelial structure of the skin in large patches and, in sloughing, leaves a slow-healing wound.

Mustard gas disables horses in various ways. The damage is generally noticed about two weeks after exposure. Because battlefield horses are seldom exposed to high concentrations, respiratory troubles are not important. By the time animal-drawn trains arrive on the scene of an advance much of the gas has been dissipated. It is contained mainly in holes cupped in the mud of roads and shellholes and, strangely, upon the leaves of trees, whence it settles down upon the backs of horses sheltered under them. Three weeks after a mustard-gas attack, horses lie in low places at the risk of having their sheaths slough off in great patches a month later. The gas derives its name from an odor resembling that of oil of mustard. It has no other claim to the name.

Treatment of heredodegenerative disorders

A continuing challenge to neurology

Walter O. Klingman, M.D.

It is with great personal pleasure that the officers and members of the Board of Trustees of the American Academy of Neurology welcome you here in Boston where so many of the membership have had their introduction to the field of neurology under numerous able and outstanding men. The attendance at this meeting, the scope and volume of the program presentations, the enrollment in the special courses conducted prior to the scientific meeting, and the number and caliber of scientific exhibits and demonstrations all bear witness to the fact that this Academy has progressed well beyond its organization period.

The American Academy of Neurology is now faced with the need of providing for development and expansion in a careful and considerate fashion, keeping our aims and goals in mind, being realistic and perhaps venturesome at times, and evaluating the increasing demands of a rapidly expanding organization.

This is the first meeting where a section activity of the Academy is included. In less than a year's time the Section on Neurochemistry has reached a point far beyond expectation. Furthermore, the large number of presentations that warranted program participation has made it necessary for the program committee to schedule scientific sessions operating concurrently. These features of the 1957 meeting undoubtedly represent the total needs of a rapidly expanding membership and also reflect the increasing importance of the role that neurology is playing in American medicine. Not only is this true of neurology in this country, but also of neurology the world over, judged by the growth in distribution of NEUROLOGY, the official journal of the American Academy of Neurology, to all parts of the globe.

This progress seems to vindicate the vision and hopes of the founders of the Academy. No doubt, it also creates some consternation among a few of the membership who may prefer restriction of these activities. However, we must meet the demands of the times realistically and be experimentalists sufficient to lead this developmental phase with well maintained guidance and control. The membership must have confidence and faith in the officers and trustees to guide this expansion of activity and to assure that this progress continues.

This preamble leads in a fitting manner to the topic of my address to you. It consists of a preliminary report of a venturesome experiment, but at the same time it expresses a cautious, experimentalist approach similar to that which faces the Academy in its expansion. In this instance, it represents a two-year venture in a therapeutic approach to the continuing problem and need of therapy for a large segment of neurologic disorders, the degenerative disorders, for which in the past we have had little to offer beyond recognition of the disorder.

In this two-year venture, observations made in the course of investigations of disorders of other character, in which metabolite deficiencies occurred, offered sufficient background to study some of the degenerative disorders. From it has emerged unanticipated degrees of change sufficient to encourage further efforts

From the Univeristy of Texas Medical Branch, Galveston, Texas.

Presidential address delivered at the ninth annual meeting of the American Academy of Neurology, Boston, April 24, 1957.

toward more specific therapy, as well as determination of etiology and pathogenesis as it applies to some of the so-called degenerative disorders of the nervous system, with or without the hereditary consideration. In this study a selection of two degenerative groups was made, the nuclear amyotrophies and muscular dystrophy. The selection was based upon degenerative and hereditary features that both present, particularly because of similarities in metabolic failure.

The nuclear amyotrophies represent a large group of clinical syndromes, depending upon location of the nuclear changes. They include such disorders as progressive muscular atrophy, the infantile forms of spinal muscular atrophy of Werdnig-Hoffmann and of amyotonia congenita of Oppenheim, amyotrophic lateral sclerosis, primary hereditary ataxia, progressive bulbar palsy, progressive chronic ophthalmoplegia, familial spastic paralysis, and Charcot-Marie-Tooth peroneal atrophy. The other group, considered under the myopathies, was progressive muscular dystrophy.

These disorders are characterized by two features. One is the early breakdown or degeneration of ganglion cells, such as occurs in the nuclear amyotrophies, and faulty development or early breakdown of muscle tissue, which is seen in the myopathy group. The second feature in practically all of these disorders is the high incidence of a genetic factor, manifesting itself as a familial disorder or being transmitted from generation to generation, sex-linked in some instances, recessive or dominant in the mode of transmission in others. Another common feature that these disorders display is evidence of faulty or immature tissue development in infancy or even in the intrauterine period, or faulty breakdown of ganglion cells or muscle cells occurring after what appears to be a normal state of health for many years after birth.

Our particular interest in these categories of disorders was aroused by studies relating to the role that ions or electrolytes play in metabolic or enzymatic activity associated with the paroxysmal disorders. In these investigations we became more and more aware of the role that certain ions play in metabolic activity of neuronal and other tissue. In recent years we have been impressed by the role that magnesium played in metabolic derangements and have already reported on clinical features associated with magnesium depletion states.[1] In this, our attention was attracted by the alterations of nerve-muscle unit activity in which magnesium seemed to have a relation to tremors, muscle twitchings and jerkings, weakness, and ataxia, aside from actual seizures states, states of clouded consciousness, or toxic exhaustion psychotic manifestations.

In reviewing our studies, it appeared that influencing nerve and muscle function in such disorders as the nuclear amyotrophies and dystrophies might conceivably alter or overcome defects which existed in these disorders due to lack, deficiency, or inability to metabolize critical ingredients or metabolites. Possibly the derangement of metabolic disorder might be corrected, reversed, or stabilized by supplying necessary enzymatic or other biochemical factors similar to those encountered in magnesium depletion states. We also assumed the presence of an inherent, genetic factor in these altered metabolic states. In the previous studies, magnesium seemed to play a necessary role in the completion of biochemical functions in both nerve and muscle tissue. Furthermore, the metabolic role of magnesium and other ions appeared to have some relation to the gene factor present in heredodegenerative disorders.

Such a concept should not be so unusual in view of our present recognition of the important role that specific vitamins play in the nervous system in deficiency states, such as pellagra, beriberi, and the anemias where there may not only be a true deficiency, but where restoration and maintenance of certain nerve cells and pathways depend upon a continuing supply of many times the normal concentration of a metabolite.

By a somewhat similar but a differing mechanism, there has been a growing approach to another nervous system disorder, hepatolenticular degeneration or Wilson's disease, which gives evidence of linkage with abnormal copper metabolism and other metabolic defects. In this disorder, there is impairment of normal blood serum ability to bind copper, presumably by a plasma protein constituent, ceruloplasmin, due to a deficiency of this particular plasma protein. As a result, there is

too much free or unbound copper present in the blood and urine. Just how these factors enter into production of the lesion is not well understood. Yet the copper and amino acid metabolic derangement in Wilson's disease seems quite definitely characteristic in the affected individuals and often in others of the family.[2]

Inasmuch as nerve cell biochemical processes are intimately related to the proper function of intracellular enzyme systems, which usually require a step-by-step completion of their biochemical assignment in normal cell life, failure to carry these processes through to metabolic completion seems to offer many opportunities where intracellular activity might be altered or break down. The biochemical processes are not necessarily confined to intracellular activities, but may also be critical for tissue membrane or extracellular activity.

Nucleoprotein activity and phosphorylation stand out as prominent factors in nerve cell activity. What is known of genes is that they are complex nucleoproteins having the properties of self duplication and ability to mutate or to alter biochemical activity. Present day concepts, supported by strong suggestive evidence, indicate that genes direct development and other functions intimately related with the biochemical activities of the organism during embryonic development, or even if development seemingly has reached a normal or complete state.[3] Preliminary deductions regarding muscular dystrophies seem to indicate that one is dealing with a disorder which, in all likelihood, is a congenital defect rather than a degenerative change later than that occurring after embryonic life.[4]

Recent experimental work on gene activity indicates that a biochemical factor is responsible for producing differentiation of cells. The factor identified in living tissue is ribonucleic acid. In further experimentation this factor was proved to have the ability to initiate cell differentiation if ATP (adenosine triphosphate) was added. Tagged ribonucleic acid protein was shown to be taken up by the differentiating cells. This suggests that specific types of ribonucleic acid may be the inducers of specific proteins, particularly enzymes, which make cell types differ from each other. There are additional clues that, as tissues differentiate and mature in the embryo, they may elaborate substances which have the ability to inhibit their still undifferentiated cells from following in the same pathway of specialization.[3]

In this fashion there seems to be a very intimate relation with biologic activities, once the organism tissue becomes complete and is apparently normal. However, the gene direction of biochemical activities or future biochemical activities and events results in altered biochemical states. This reduced or altered relationship results in misdirected activity and the result is a so-called degenerative disorder. Gene activity may also be responsible for electrolyte or ion disorder involved in metabolism. An example of this is found in familial periodic paralysis, a dominant hereditary affliction due to a potassium metabolic disorder. In some instances of this disorder there may also be muscular atrophy, in addition to episodes of transient paralysis.

Such recognized biochemical abnormalities due to genetic mechanisms are thought to cause specific effects which will result in abnormalities, such as an abnormal binding of potassium in family periodic paralysis or, in hepatolenticular degeneration where a deficiency may exist in a particular protein's ability to bind copper, aside from an alteration which influences the kidney threshold for certain amino acids.

Therefore, considerable seems to be known or observed or postulated for such conditions that permit one also to apply these concepts to other hereditary disorders or degenerative diseases as they affect nerve or muscle tissue, such as in the nuclear amyotrophies or in the myopathies. In the dystrophy group one may actually be dealing with a congenital defect which later in life is manifested as a tissue metabolic alteration or breakdown or degeneration. This same characteristic may well apply to some of the other hereditary degenerative disorders of the nervous or muscular systems, whereas formerly this feature was explained on the basis of abiotrophy.

In a somewhat similar light, we understand better how exogenous toxins, such as arsenic, influence nervous system metabolic activity by a blocking effect or competition for a critical substance necessary to maintain normal neural

structure activity; as a result, a degenerative change in the nature of a neuropathy occurs.[5]

At a previous Academy meeting, a new kind of attack on epilepsy was launched upon findings suspecting a basic defect in body chemistry, with the hope that regular intake of ingredients would lead to the correction of a metabolic defect and control of epilepsy by maintaining and adequate intake and maintaining in nerve cells two important biochemicals, glutamine and asparagine. In these efforts was represented the contribution of amino acids for utilization of a supply of energy for the deficient nerve cell in the hope that this would influence a biochemical lesion in the brain that causes epilepsy. By correcting a deficit of critical ingredients found in nerve cells in areas from which seizures were arising, it was hoped that control of convulsions by such means might be obtained, rather than by medications which function by depressing nerve cell activity. The biochemicals would, in this sense, assist in replacing specific nerve cell deficiency that leads to convulsions.[6]

We were particularly impressed by the role that ions played in metabolic processes. Inasmuch as one of our interests originally concerned magnesium, we began to interest ourselves even more in its relationship to other metabolites or the combinations with other metabolites that might constitute critical factors in maintaining healthy nerve or muscle cell activity. The fact that many enzymatic reactions were accelerated by sufficient presence of certain ions, or where completion of biochemical processes did not occur except in the presence of certain ions or other metabolites, led to evaluation of the effects of supplying ingredients to individuals who had degenerative nervous system disease. Important in the functioning of nerve and muscle cells seemed to be two major ones, whereby favorable utilization of carbohydrate metabolism and the adenylic system might indicate that critical ingredients involved might also provide key metabolites to restoration and maintenance of function, provided tissue changes had not already reached irreversible stages.

In this evaluation it became apparent that, in the process of the breakdown of glucose, high energy phosphate bonds were created as a result of the adenylic system activity. In this it also became apparent that the adenylic system participated in vitamin action and otherwise provided energy for muscle contraction and relaxation. The universal source of the immediate energy for activity of nerve and muscle tissue, as well as other tissues, was adenosine triphosphate (ATP). It also entered into the activities that pertain to nerve impulse conduction, contractility of muscle and secretion. Considered important by many investigators is its relationship to the process whereby production of acetylcholine activates nervous tissue. It had already been recognized that the action of ATP was markedly potentiated by the administration of magnesium. It had also been shown that in muscle glycolysis some phosphate group transference was accelerated by sufficient presence of magnesium and cobalt ions and other ions.[7]

Elaborate metabolic processes are present in carbohydrate breakdown, and the presence of certain metabolites is necessary for the completion of the metabolic reaction. These include, among others, adenylic acid (precursor of ATP), magnesium or manganese ions, and thiamine and methionine. Other enzymes involved in muscle phosphorylation are the phosphokinases. These all have the common property of requiring the presence of magnesium. Thiamine is one of the phosphokinases. ATP also plays a role in formation of creatine, which could be important in muscle metabolism in that creatine phosphate present in muscle acts as a reservoir of available energy. In the phosphate energy reactions a dependency could exist upon the concentration of an ion, magnesium, to form the complexes relating to ATP, and, conceivably, this might be an important factor. In the complete absence of magnesium the enzyme factor has been known to remain inactive.

Person in 1955[8] had postulated that in progressive muscular dystrophy there was an inherited or acquired derangement in the combination between the iron-actomyosin and ATP which causes muscle contraction. An explanation for the reduction in creatine in progressive muscular dystrophy was secondary to reduced or available ATP, according to his concept. He likewise referred to the need for presence of iron for the phosphorus uptake of actomyosin and indicated that a part of the iron in muscle

is in an as yet unknown type of combination with the actomyosin. Szent-Gyorgi[9] could initiate muscle contraction with the action of ATP. He further reported that Reinhold and Kingsley[10] contended that there is a reduction of the absolute quantity of ATP per gram of muscle in muscular dystrophy. Person felt that, in the final analysis, the creatine and phosphocreatine disappear more quickly than the ATP; he felt that the essential factor was the reduced total content of ATP in muscle and that active muscle is replaced by fat and connective tissue eventually as a result of these alterations.

Nerve and muscle tissue have factors of metabolic importance in common in their metabolic activities. In the nuclear amyotrophies, as well as in muscular dystrophy, progressive muscular atrophy, amyotonia congenita, and very likely also myasthenia gravis, lack of derangement of proper metabolic activities is under suspicion. Detecting the key defects in these abnormal metabolic processes or derangements offers some clue, leading to a correction of the biochemical lesion or proper maintenance or sustained physiologic function by such correction. These may involve processes intimately related to intracellular enzyme systems or cell surface or membrane activities. Many of the disorders have genetic factors involved, suspected as being nucleoproteins in nature. Carbohydrate glycolysis and nucleoprotein linkage in phosphorylation necessary for proper nerve-muscle function seem to be well established.

Because of these considerations and because time does not permit us to go into the complexities of the involved biochemical factors, our interest in key metabolites and ion activity led to an effort over a two-year period to note changes occurring in hereditary degenerative disorders by supplying some of the involved metabolites, making more ATP available, catalyzing phosphorylation, and influencing energy transfer exchanges which govern physiologic processes in which carbohydrates, nucleotides, and ions are involved. This has resulted in most encouraging clinical responses. The role of magnesium was respected, but it was difficult to find a magnesium salt which would be assimilated until magnesium gluconate was found to have such property. The most active phosphorylation needs are known to exist in brain tissue as well as in muscle tissue. Supplying added sources to contribute to improvement of function of nerve and muscle tissue was attempted by the administration of 1) adenylic acid in the form of adenosine-5-monophosphate, commercially available as My-B-Den; 2) thiamine hydrochloride orally, or Beta-methiscol, for its thiamine, methionine, and choline content; and 3) magnesium gluconate. Choline was felt to be desirable for muscle metabolism. These were given in the following manner: My-B-Den (sustained action form) was given in a 1 cc. intramuscular injection in the gluteal muscles three times per week. Thiamine was given as a single 100 mg. dose orally each day, or Beta-methiscol was given in 1 tablespoonful amounts three times daily. Magnesium gluconate was given in 250 mg. tablet form three times daily. A rectal suppository form of My-B-Den has also been made available for use in young children. Its effectiveness in maintaining blood ATP levels, however, is only approximately 45 to 50 per cent as efficient as giving My-B-Den by the intramuscular method.

The accompanying tables are self-explanatory. Table 2 shows the blood and magnesium serum levels of patients who have been on the treatment program, as well as some untreated cases.

Over 100 cases of nuclear amyotrophy and muscular dystrophy have been observed for the effect of the therapy approach outlined. The most surprising in respect to improving

TABLE 1
INCREASE IN BLOOD ATP — MG. PER CENT

50 mg. My-B-Den	*50 mg. My-B-Den with Mg. Cl_2*	*300 mg. Magnesium gluconate*	*50 mg. My-B-Den with 300 mg. Magnesium gluconate*
0.1	0.3	0.9	1.1
0.5	0.6	0.6	0.8
0.0	0.4	0.6	1.2
0.2	0.1	0.3	1.1
KC 0.1	0.2	0.7	1.3
0.2	1.0	BB 1.2	0.5
0.0	0.6	0.5	1.2
LK 0.2	0.4	0.3	0.0
BB 0.0	0.1	0.9	0.4
0.0	0.5	LK 0.4	0.7
		0.0	0.0
		KC 0.9	0.0

TABLE 2

Name	Dx	Magnesium	AA	ADP	ATP	Total Purine	
C.U.	MS	1.69	0	0	5.9	11	T
M.D.G.	ALS	1.73	0	0	7.2	18.7	B-M
P.H.W.	CMT	1.73	0	0	5.2	13.3	T
G.H.	CMT	1.73	0	0	6.4	11.2	T
H.R.	MY	2.30	0	0	4.9	19.0	O(U)
S.F.	MD	1.69	0	0	7.8	16.1	T
J.S.	MD	1.56	0	0	3.0	10.9	O(U)
G.F.	MS	2.15	0	0	5.4	8.9	T
R.B.	MD	1.64	0	0	4.9	7.8	O(U)
B.K.	HA	1.64	0	0	5.4	13	B-M
R.D.	HA	1.52	0	0	6.4	9.6	B-M
B.S.N.	ALS	1.89	0	0	8.0	14.3	B-M

Attention is called to the elevation in blood ATP and total purine levels in this series receiving ASMP (My-B-Den), magnesium gluconate, and either thiamine or Beta-methiscol. Table 1 shows the striking differences obtained by various combinations and dosage of My-B-Den and magnesium chloride or magnesium gluconate, but without either thiamine or Beta-methiscol. The cases marked U were cases of neuromuscular disorders receiving other medications. The ATP levels are in mg. per cent. The cases receiving thiamine are designated by T and those receiving Beta-methiscol are designated B-M.

or maintaining function has been the muscular dystrophy group. Amyotrophic lateral sclerosis likewise has presented clinical courses not usually observed in untreated or empirically treated cases. Several in this group have made apparent dramatic recovery but also have given evidence of continued need of supportive or maintenance courses at intervals when indications of recurring signs or symptoms appear. In the group of nuclear amyotrophies have been included cases of family hereditary cerebellar ataxia and Friedreich's ataxia with variants, such as ophthalmoplegia, where improving function has been noted. One case of nuclear amyotrophy with bulbar involvement of severe degree with difficulty in deglutition, swallowing, and saliva control, intrinsic muscle atrophy of the tongue, and weakness of the erector capitis groups showed progressive improvement to the extent that the patient requested permission to return to his former full-time occupation.

Approximately 25 to 30 per cent of the cases continued to have a progressive downhill course, and it may well be that other key metabolites are involved. Also, at the beginning of our study, cases were included regardless of the stage or phase or duration of the disorder. It would be unnecessary to state that, if any influence was brought to bear upon these states, already irreversible changes would not be altered. In the latter period of study, cases were selected in the early stages of the disorder but where the clinical and other findings were in support of the diagnosis. In a number of instances, however, trial periods were given in advanced cases, purely because of the humanitarian aspects of the care and management of such cases. The decision of trying out the proposed therapy was left to the patient and the family, both being fully acquainted with the fact that it was a therapeutic trial.

Work in continuation of this approach is being carried on, considering other defects that may be responsible. Improved tissue and fluid assay methods, neural and muscle tissue culture, and model observations give promise of help in determining factors to be taken into consideration. Precursors and other bases are also being studied for evidence of deficiency or intermediary interruption of their biochemical or metabolic assignment.

The information presented must not be misconstrued as something equivalent to a cure. It is presented in the nature of preliminary observations upon the effects of the course of some degenerative disorders over a two-year period. It could well be that merely making more ATP available will only sustain or assist function temporarily and that actual correction of the degenerative process may require other assistance.

This paper is in the nature of a preliminary report in respect to meeting a continuing challenge that is presented in neurology by the

hereditary degenerative disorders. An experimental approach to such problems has been presented, with the underlying postulates. It is our hope that, with the enthusiasm and interest which this Academy has provided, the potentialities of unraveling the mysteries of so many disabling nervous system disorders can be realized by many in this organization, through the concerted effort of neurophysiologists, neuropathologists, neuropharmacologists, chemists, and the venturesome experimentalists. Nihilism should have no place in this organization. Neurology cannot afford to remain static in regard to these disorders.

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Epilepsy varies not only as to its form and degree, but as to the duration of the paroxysms, and the time of its return. Accounts of great varieties in these respects, might be adduced from numerous authors. The fits may last for a few seconds or minutes, or for many hours. In the case of a girl twenty years of age, the paroxysms, though not very strong, always lasted for fourteen hours. The ordinary duration of these attacks, is from ten to twenty minutes, when, the disease having arrived at its height, the respiration becomes more slow and easy, and the other symptoms disappear.

John Cooke in *A Treatise on Nervous Diseases*, published in 1824.

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EFFECTS OF MAGNESIUM SULFATE, CALCIUM GLUCONATE, POTASSIUM CHLORIDE AND AMMONIUM CHLORIDE ON THE URINARY EXCRETION OF MAGNESIUM IN PATIENTS WITH IDIOPATHIC CARDIOMYOPATHY AND CONGESTIVE HEART FAILURE*

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The effects of magnesium sulfate, calcium gluconate, potassium chloride and ammonium chloride on the rate of urinary excretion of magnesium and other electrolytes have been studied previously. However, most of these studies were confined to acute experiments on normal animals or man and the results are contradictory. This study was performed on a daily basis in 2 patients with chronic congestive heart failure and one control subject followed continuously for several weeks.

Material and Methods. Three adult females at the Charity Hospital in New Orleans were studied. Subject B.S., aged 44 years, weight 65 kg., with psychoneurosis, served as a control. Patient M. M., aged 49 years, weight 50 kg., had idiopathic cardiomyopathy and chronic moderately severe congestive heart failure. Patient O. J., aged 56 years, weight 70 kg., had idiopathic cardiomyopathy and chronic severe refractory congestive heart failure. Both patients with heart failure were on maintenance digitalis. All of the subjects were under metabolic study conditions for a month before the various salts were given. They were kept in bed and fed identical diets containing approximately the following amounts of electrolytes per day: Na, 1 gm.; Cl, 1 gm.; K, 2 gm.; Mg, 0.25 gm.; and Ca, 1 gm. Daily urine specimens were collected from approximately 8 a.m. to the next 8 a.m. A period of 6 to 10 days served as control. Twelve milliliters of 50% $MgSO_4$ were given intramuscularly at 10 a.m. on the first day of the experimental period; 30 ml. of 10% KCl were given orally in divided doses at 10, 11 and 12 a.m. on the third day; 40 ml. of 10% calcium gluconate in 500 ml. of 5% glucose were given intravenously from 10 a.m. to 3 p.m. on the fifth day; and 9 gm. of NH_4Cl were given orally in divided doses at 8 a.m., 2 p.m. and 4 p.m. on the seventh day. Sodium and potassium were determined by conventional flame photometry. Magnesium and calcium were also determined by flame photometry (Zeiss PMQII with double monochromator) according to the method described by MacIntyre[11]. Chloride was measured by the method of Schales and Schales[16].

Results. CONTROL PERIOD. The daily rates of urinary excretion of Mg and other electrolytes in the 3 subjects during the control period are summarized

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TABLE 1.—THE MEAN, STANDARD DEVIATION AND RELATIVE VARIATIONS* OF URINARY EXCRETION OF WATER AND ELECTROLYTES DURING THE CONTROL PERIOD†

	Mg	*Ca*	*K*	*Na*	*Cl*	*Volume*
Control Subject BS						
Mean ± SD (mEq. or ml. per day)	5.60 ± 1.78	5.66 ± 2.32	35.6 ± 10.0	38.3 ± 11.1	46.5 ± 15.3	1124 ± 302
Relative variation (percent)	32	42	28	29	33	27
Patient MM (moderate CHF)						
Mean ± SD (mEq. or ml. per day)	4.78 ± 1.70	0.35 ± 0.16	34.3 ± 8.0	9.6 ± 3.4	31.1 ± 10.0	915 ± 288
Relative variation (percent)	36	46	23	35	32	31
Patient OJ (severe CHF)						
Mean ± SD (mEq. or ml. per day)	2.52 ± 0.66	0.30 ± 0.12	18.1 ± 1.4	25.4 ± 22.5	19.8 ± 16.8	495 ± 118
Relative variation (percent)	26	24	7	88	85	24
Normal Range[3] (mEq. or ml. per day)	1.80–23	2.50–21	28–126	61–338	63–380	430–4100

* Relative variation is expressed as percent $\left(\frac{S_X}{\bar{X}}\right)$, where S_X and $\bar{X}$ are the standard deviation and the mean respectively.

† Control period was 10 days for Control Subject BS and Patient MM (moderate congestive heart failure) and 6 days for Patient OJ (severe congestive heart failure).

in Table 1. The normal ranges for normal subjects under ordinary diet and activity are shown for comparison (Elliot[7]). In the 3 subjects studied the rates of urinary excretion of Na and Cl were all below the normal range and the rate of excretion of Ca was also unusually low in the patients with congestive heart failure. The standard deviations expressed in percentages of the respective means [or relative variations (Bancroft[2])] for all the para-

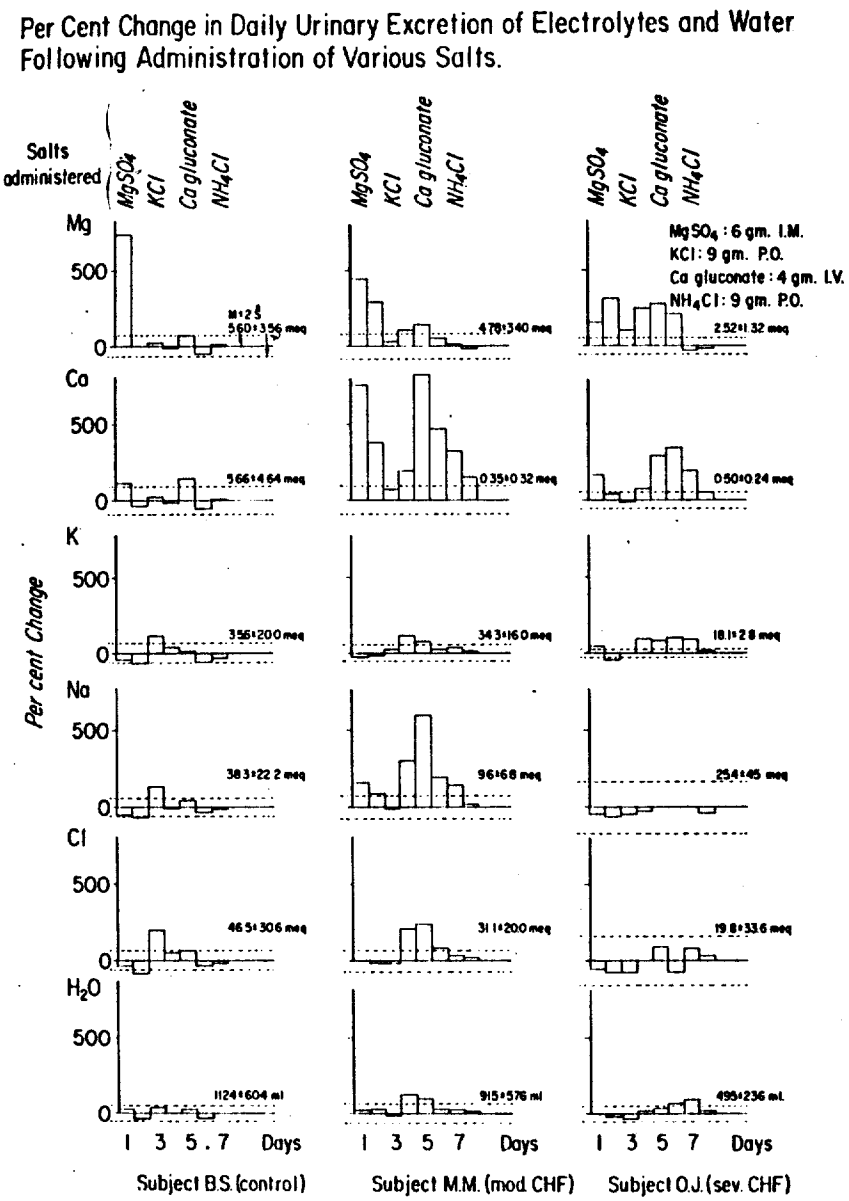

Fig. 1.—Percent change, from the respective means during control periods, in daily urinary excretion of electrolytes and water following administration of various salts. The broken lines denote 2 standard deviations (95% confidence limit) graphed as percentages of the respective means during the control period (see Table 1). The absolute values for the means and 2 standard deviations are shown. The control periods were 10 days for control subject B. S. and Patient M. M. and 6 days for Patient O. J.

meters were 23 to 46% except in the patient with severe congestive heart failure (Patient O. J.) in whom the relative variations for Na and Cl were 88% and 85% respectively and that for K was 7%.

INTRAMUSCULAR ADMINISTRATION OF ($MgSO_4$.) The rate of urinary excretion of Mg on the day of administration of $MgSO_4$ was significantly (over the 95% confidence limit) increased in all the 3 subjects, the increments being 740% in Subject B.S. (control subject); 440% in Patient M. M. (moderate congestive heart failure) and 150% in Patient O. J. (severe congestive heart failure). The response continued to the postmedication day in the 2 patients with congestive heart failure, the excretion remained 300% higher than the respective means of the control period for both patients. The rate of urinary excretion of Ca was also significantly increased in all the 3 subjects. Patient M. M. had the highest increment, being 750% above the mean of control period on the medication day and 360% above the mean on the postmedication day. Excretion of Na was increased to 150% above the mean of control period in Patient M. M. only. Excretion of K, Cl and H_2O was not significantly altered (Fig. 1).

INTRAVENOUS ADMINISTRATION OF CALCIUM GLUCONATE. The rate of urinary excretion of Ca was significantly increased in all subjects after administration of the calcium gluconate, the increments being greater and more prolonged in the 2 patients with congestive heart failure. The effects of Mg excretion were similar to that of Ca but less marked. Excretion of K was increased in both patients with congestive heart failure but not in the control subject. Significant increases in the rate of excretion of Na, Cl and H_2O occurred only in patient M. M. (Fig. 1).

ORAL ADMINISTRATION OF KCl. The rate of urinary excretion of K was significantly increased on the day the KCl was administered in Subject B. S. (control subject); and on the postmedication day in Patients M. M. and O. J. Excretion of Mg was increased only in the patients with congestive heart failure, being greatest on the postmedication day. Significant increases of Ca excretion was seen in the patients with congestive heart failure only on the postmedication day. The rates of excretion of Na and Cl were significantly increased in control Subject B. S. on the medication day, in Patient M. M. on the postmedication day and were not changed in Patient O. J. A significant increase of H_2O excretion occurred in Patient M. M. only on the postmedication day. (Fig. 1).

ORAL ADMINISTRATION OF NH_4Cl. The rate of urinary excretion of Cl and Mg was not significantly affected by the oral administration of NH_4Cl in all the 3 subjects. Excretion of Ca was significantly increased in both patients with congestive heart failure on both the medication and postmedication days. Excretion of K was significantly increased only in Patient O. J., whereas excretion of Na was significantly increased only in Patient M. M. (Fig. 1).

Discussion. A decrease in the rate of urinary excretion of K following the administration of $MgSO_4$ has been reported previously (Barker, Elkinton and Clark[3], Heller, Hammarsten and Stutzman[9], Jabir, Roberts and Womersley[10], Womersley[20]). It has long been known that aglomerular fish excrete Mg by renal tubular secretion (Bieter[4]). Renal clearance of Mg had been found to exceed glomerular filtration rate during constant infusion of large amounts of Mg in dogs (Elkinton[6]) and also in a patient with tubular alkalosis (Schales and Schales[16]). Furthermore, direct evidence of tubular secretion of Mg had been shown in dogs by the stop flow technique (Ginn *et al.*[8]). Therefore, the concept of tubular secretion of Mg in a manner similar to that of K was suggested; and the findings of a decrease in the rate of excretion of K following in-

fusion of $MgSO_4$ were explained on the basis of competition for tubular excretion between Mg and K ions (Heller, Hammarsten and Stutzman[9]). However, infusion of $MgCl_2$ was found to be associated with an increase, rather than a decrease, in K excretion (Samiy, Brown and Globus[11]) and the administration of KCl was not consistantly accompanied with decrease in the rate of urinary excretion of Mg (Jabir, Roberts and Womersley[10]). In this study, the excretion of K was found not to be significantly influenced by the administration of $MgSO_4$. Furthermore, the excretion of Mg was increased following the administration of KCl in 2 of the subjects. Therefore, tubular secretion of Mg might not follow the same pattern as that of K, if there is any tendency for this to exist.

The increases in the rate of urinary excretion of Mg and Ca following the administration of either of the two ions has been reported by others (Ardill *et al.*[1], Barker, Elkinton and Clark[3], Walser[18], Womersley[20]). The theory of "common renal tubular reabsorption mechanism" (Samiy, Brown and Globus[15], Wolf and Ball[19]) for both of the bivalent cations seems to be true. In the 2 patients with chronic congestive heart failure, the effects were even more marked and prolonged than in the control subject. This suggests a decreased total reabsorption capacity for the cations in the patients with chronic congestive heart failure.

Excretion of Na, Cl and H_2O was not significantly altered by either $MgSO_4$ or calcium gluconate in this study except in Patient M. M. She also had the greatest clinical response to diuretics (Yun, Lazzara and Burch[21]). The prolonged low salt diet might have contributed to the failure to respond to these ions.

No consistent effect on urinary excretion of Mg following the administration of KCl has been reported (Burch *et al.*[5]). In this study, Mg excretion was increased following the administration of KCl only in the patients with chronic congestive heart failure. The relative increases of the rapidly exchanging Mg mass in the patients with chronic congestive heart failure, as reported from this laboratory (Burch *et al.*[5]) might have contributed to this difference. The diuretic and natruretic effects of KCl (Pitts[13]) were seen in Subject B. S. (control subject) during both the day of KCl administration and the next day and also in Patient M. M. (moderate congestive heart failure) in the postmedication day. Patient O. J. (refractory congestive heart failure) showed no response. This conformed well with the clinical state.

Increase in the rate of urinary excretion of Mg and Ca following the administration of NH_4Cl has been observed previously (Jabir, Roberts and Womersley[10], Martin and Jones[12]). Increase in the diffusible fractions of these ions due to acidification had been suggested as an underlying mechanism. In this study, the rate of urinary excretion of Mg was not changed in any of the 3 subjects whereas the rate of urinary excretion of Ca was significantly increased in 2 of the subjects (Fig. 1). Also, following acetazolamide the rate of urinary excretion of Ca had been found to increase markedly whereas the rate of excretion of Mg was somewhat decreased (Barker, Elkinton and Clark[3]). Therefore, acidification either by NH_4Cl or by acetazolamide influenced differently the rates of urinary excretion of Mg and Ca, and must not be the sole cause for the change in the rate of urinary excretion. Tubular handling of available cations to maintain electroneutrality in the urine following the administration of NH_4Cl might also have contributed to the observed phenomena.

Summary. 1. The rate of urinary excretion of Mg and Ca was increased by parental administration of either of the two ions in a control subject and in 2 patients with chronic congestive heart failure. In patients with con-

gestive heart failure, the effect was prolonged and continued into the postmedication day.

2. The rate of urinary excretion of K was not decreased following parental administration of $MgSO_4$ in any of the 3 subjects. The rate of urinary excretion of Mg was increased following oral administration of KCl in the patients with congestive heart failure on both the day of medication and the post-medication day. The physiologic significance on tubular secretion of Mg was discussed.

3. The response of the patient with moderate congestive heart failure and the control subject was greater than that of the patient with severe congestive heart failure with an increase in the rate of urinary excretion of Na, Cl and H_2O following the administration of various ions.

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(Interlingua on page 192)

J. Pharmacol. & Exper. Therap. 40:65-70
Sept. 1930.

31a

STUDIES ON CALCIUM

I. SOME COMPARATIVE PHARMACOLOGIC EFFECTS FOLLOWING THE INTRAVENOUS INJECTION OF CALCIUM LACTATE AND CALCIUM GLUCONATE IN UNANESTHETIZED DOGS[1]

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From the Hull Physiological Laboratories of the University of Chicago

Received for publication June 21, 1930

It seemed advisable to determine by a series of hemodynamic and respiratory studies the comparative effects of calcium lactate and calcium gluconate upon unanesthetized dogs when injected intravenously. Because it is well known that anesthesia renders animals much less sensitive to small doses of drugs (1) and may even reverse results obtained in the absence of anesthesia (2), (3) and because, clinically, anesthesia is not used during this type of medication, it was desired to obtain results under conditions in which anesthesia did not enter as a factor.

METHODS

Apparently healthy dogs of average size (8 kgm.) were used. Under very light ether anesthesia and with ordinary aseptic precautions, the carotid artery on one side was cannulated. The neck was bandaged with the cannula left in place and the dog was allowed to recover from the ether anesthesia. One hour later the dog was injected subcutaneously with ¼ grain of morphine sulphate. After another thirty minutes had elapsed, the dog was placed gently on the table and the cannula connected with the usual mercury manometer. A pneumograph was strapped to the chest and attached to a tambour for registration of the respiration. After taking a normal blood pressure and res-

[1] Work done under grant from Sandoz Fund.

piration record. calcium gluconate in 10 per cent solution was injected intravenously with such slowness that about five minutes were consumed in the process. About 30 cc. of the solution was all that the average 8-kgm. dog will stand without showing marked distress. This amounts to about 35 to 40 mgm. of calcium ion per kilogram body weight. Records were taken for one-half to one and one-half hours after injection. By that time all effects of the injection had usually disappeared. The dogs lay rather quietly in a room from which all extraneous stimuli were excluded as much as possible. Finally, the carotid was tied off (again under aseptic precautions) and the neck wound sewed up.

Three to five days later the same procedure was repeated using the other carotid artery this time for the registration of the blood pressure and heart rate but injecting calcium lactate instead of calcium gluconate. An equimolecular amount of calcium in the form of calcium lactate was dissolved in an equal amount of water. In this experiment it was necessary to use 1.5 grams of calcium lactate in 30 cc. of water this amount furnishing about the same number of calcium ion as does 30 cc. of the calcium gluconate, Sandoz.

The next dog was subjected to the same type of experiment but in reverse order, i.e., the calcium lactate was injected first; and the calcium gluconate was injected intravenously three to five days later.

In this manner a total of eight dogs was used, the two calcium salts being compared on one and the same dog as just discussed. It was thought that in this way a much better comparison of the two calcium salts could be made since idiosyncrasies of individual dogs were thus eliminated.

RESULTS

The most significant values for blood pressure and pulse rate are shown graphically in figures 1 and 2. It can be seen that the blood pressure rises quite promptly on injection of each calcium salt. At the end of half an hour the blood pressure following the injection of calcium lactate had almost returned to nor[illegible]. With calcium gluconate, however, the hemodynamic

effect persists and is sometimes still present one and one half hours after the intravenous administration. The pulse rate findings are interesting. With the lactate salt a very prompt slowing was obtained but the effect wore off rapidly, being almost gone at the end of half an hour. With the calcium gluconate,

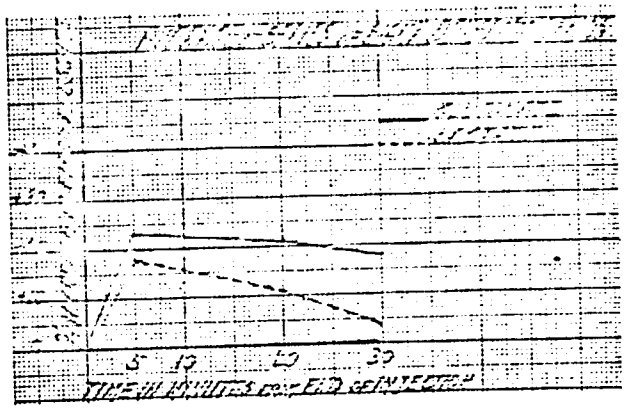

FIG. 1

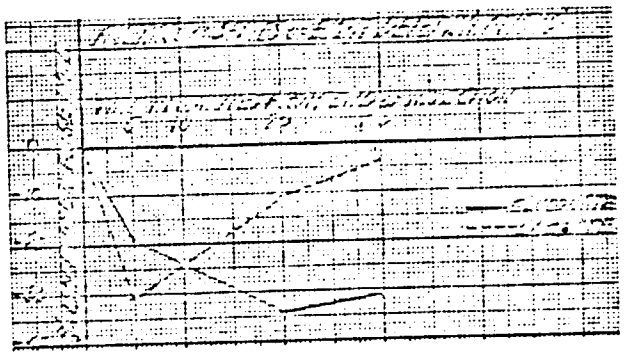

FIG. 2

however, the effect, while slower to make itself manifest, persisted a much longer time. At the end of half an hour, the pulse rate was still slowed by a third and, although it is not seen on the chart, at the end of two hours some dogs still showed a very definite bradycardia.

Another very interesting, even if somewhat disconcerting,

ture in this series was the accident of intravascular clotting, ile injecting intravenously. In one of the dogs the following opened. While beginning to inject calcium lactate solution, dog which had been very comfortable, suddenly developed lent diaphragmatic spasm and air hunger but gradually re- ered: and the experiment was terminated. A necropsy was n performed and a small ante-mortem clot was found in the ht ventricle. Another dog appeared entirely comfortable ile receiving intravenous calcium gluconate. Without any ning and at the conclusion of the injection the dog gave sev- l deep gasps and died. A large, fresh clot filled the entire t heart. In line with this rather sudden intravascular clot- g, there was considerable difficulty in keeping the blood in the nidae from coagulating—much more so than when other than lcium salts are used. This is of clinical importance as I know rsonally of two accidents that happened here in Chicago within e year. In neither case did the patient die but each developed, ile receiving intravenous calcium lactate, sudden air hunger, aphragmatic spasm, etc., so that it is reasonable to assume at some intravascular clotting had occurred. I believe that is is the only real counter-indication to intravenous calcium erapy but it is certainly a weighty one. Borbely[2] as quoted Barath observed experimentally a diminution in hemor- agic tendencies following calcium injections. Also Szentch noted in a series of over fifty deliveries a very definite de- ease in the amount of blood lost after he had put these women a course of calcium gluconate injections intramuscularly ven ante-partum. There seems to be no doubt that calcium ry definitely increases the coagulability of the blood.

Another very interesting finding and one that has been noted others (5) is the digitalis-like action of the calcium (6). Appar- ly it is a prompt and direct action on the myocardium. Arry- mias were repeatedly induced by merely giving excessive doses. ecognizable block, coupling of beats, etc., could be induced. his raises the question of whether, in desperate emergencies

[2] Borbely as quoted by Eugene Barath of Budapest in a paper not as yet published but seen by Professor Luckhardt.

with myocardial failure, intravenous calcium gluconate might not be of value while waiting for the digitalis to take hold.

Respirations are not affected in any noteworthy way by calcium injections except when *excessive* dosages are used when Cheyne-Stokes type of breathing was noted several times.

SUMMARY AND CONCLUSIONS

The actions of calcium gluconate and calcium lactate injected intravenously have been studied on the blood pressure and pulse rate and respirations in a series of 8 anaesthesized dogs.

The maximum dose for this series was found to be 35 to 40 mgm. of calcium ion per kilogram body weight. For an 8 kgm. dog this meant 30 cc. of calcium gluconate in 10 % solution or 1.5 grams of calcium lactate dissolved in 30 cc. of water.

The blood pressure was found to be elevated by the injections except when excessive doses were used when there was a pronounced drop. The effect of the gluconate salt was found to persist for a much longer time than that of the lactate.

The pulse was markedly slowed especially with calcium gluconate, with which salt the effect also persisted a much longer time.

Digitalis-like effects were noted repeatedly.

Respirations were not affected constantly. With overdosages a Cheyne-Stokes type of respiration was noted several times.

The margin between effective and toxic doses is not great.

Intravascular clotting is the greatest danger facing intravascular calcium therapy. It gives no warning and the effect can be sudden fatality.

Before closing I would like to express my great indebtedness to Professor Luckhardt in whose laboratory this work was done and whose constant guidance made it possible.

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J. Pharmacol. & Exper. Therap. 40: 71-76
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STUDIES ON CALCIUM[1]

II. URINARY OUTPUT OF CALCIUM IN NORMAL INDIVIDUALS AFTER PERORAL ADMINISTRATION OF CALCIUM LACTATE AND CALCIUM GLUCONATE

ARNOLD L. LIEBERMAN, M.D.

From the Hull Physiological Laboratories of the University of Chicago

Received for publication July 7, 1930

It is of practical value to answer for a given drug two questions: How much? When? In so far as peroral administration of calcium salts is concerned, it seems to have been taken for granted that absorption is better on an empty stomach and that the equivalent of 3 to 4 grams of calcium lactate per day is about the right dose for an adult, except in cases of extreme calcium deficiency as in hypoparathyroidism when a much larger amount must be given. It seemed worth while to gather some precise data on this subject.

METHOD

Although less than 10 per cent of peroral calcium is excreted in the urine, it was assumed that the urinary calcium would, nevertheless, serve as a fairly reliable index of the rate of the blood calcium changes. Accordingly, three healthy individuals submitted to the following procedures. As a control, the individual voided hourly from 7 a.m. to 2 p.m. He ate nothing from arising to 2 p.m. He drank a glass of water (250 cc.) hourly from 8 a.m. to 1 p.m. There were no other limitations. The next day calcium lactate was given at 8 a.m. in 250 cc. of water. Everything else was maintained the same. The following day the routine was repeated except that calcium gluconate was given in 250 cc. of water. After an interval of a day, the order of calcium salts was reversed calcium gluco-

[1] Work done under grant from Sandoz Fund.

...te was given first, calcium lactate on the second day. Each subject kept a rather detailed record of pertinent signs and symptoms such as abdominal discomfort, nausea, headaches, diarrhea, borborygmi, etc. At first, the subject was given 20 grams of calcium gluconate and 10 grams of calcium lactate but the subjective and objective symptoms (abdominal distress, vomiting and diarrhea) were so violent that the doses in this series were halved, 10 grams of calcium gluconate and 5 grams of calcium lactate being given. The powder was simply dissolved in a glass of water (250 cc.) and drunk. This procedure was then repeated with the important difference that the individual had his usual breakfast about 7:30 a.m. It was uniform each time consisting of two eggs, two cups of coffee, and one piece of toast. For the urinary determinations, Lyman's (1) method was used with only this difference: instead of washing through filter paper, centrifuge tubes were employed. This saved a great deal of time and was as accurate, by actual check, as the unmodified method. The volume of the specimen obtained each hour was recorded and the milligrams of CaO in each specimen determined.

By its very nature, urinary calcium cannot give as true a picture of the level of calcium in the blood as can an actual blood calcium determination. However, for obvious practical reasons, the calcium output in the urine was chosen as the index as to the rapidity and extent of absorption and excretion of the calcium ingested.

RESULTS

Several rather interesting points stand out in the data accumulated. Figure 1 shows graphically the volume of urine. It is the average of 18 determinations each on the calcium gluconate and calcium lactate with 9 controls. As can be seen, ingested calcium salts cause a very definite diuretic effect. Whereas at 2 p.m. the controls showed a volume of only 178 cc., the average volume for the gluconate salt was 360 cc.—a volume twice as great. However this action can be explained on the mere physical basis of the greater volume of water being necessary to put out a larger amount of calcium present. Of course, the individuals here

were presumably healthy so that this would have nothing to do with experiments like those of Baroth and Gyurkovich (2) who showed that calcium salts cause a diuresis and a diminution of the albuminuria in nephritic edema. In these cases Blum (3) may be right in saying that the action is due to the dehydration of the blood colloids with resulting hydremia and diuresis.

Figure 2 shows the amount of CaO in milligrams that appeared in the urine during the period of observation. It will be seen that the individual puts out normally somewhere between 2 and 5 mgm. of CaO hourly. After the ingestion of the calcium salt

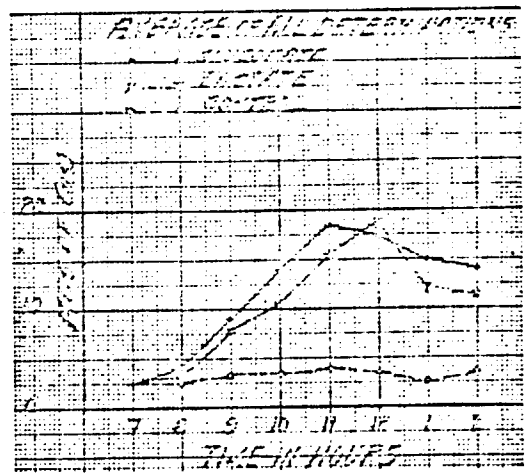

FIG. 1

there is a fairly smooth curve upwards with a maximum attained in some four hours. Then there is a beginning decrease. There is very little to choose between the two salts although a little more of the gluconate salt is recovered. However, there is a vast difference in the subjective symptoms. The gluconate was fairly well tolerated giving rise chiefly to rather annoying borborygmi and some abdominal distress. The lactate salt in equimolecular amounts gave rise to a very disagreeable headache and quite stormy bowel movements accompanied by a good deal of bowel spasm. Altogether, about 5 to 8 per cent of the calcium given was recov-

ed in the urine which is about the amount usually quoted in the terature. A much more interesting and very instructive finding summarized in figure 3. As can be seen gluconate given on n empty stomach reaches a maximum within two hours and then gins to decrease. When given after a meal it takes longer for it appear in any amount in the urine but six hours after ingestion e curve had not yet begun to slope downwards. As a net result uch more of the calcium gluconate is utilized by the body.

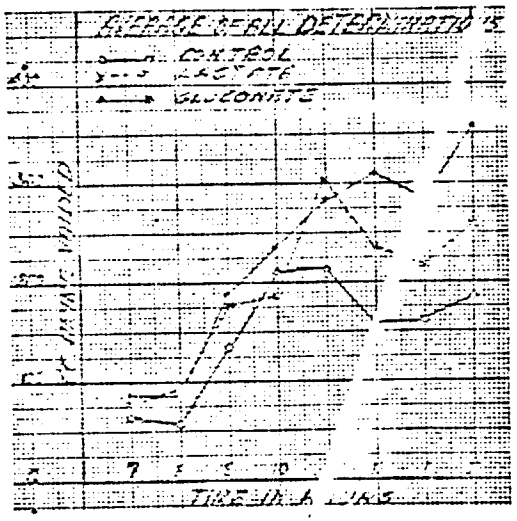

FIG.

Also, there was almost complete freedom from subjective symptoms. Except for the slight discomfort right after taking the material, one almost forgot that anything had been ingested. It seems, in this series at least, that there can be no question that better and longer absorption was obtained by taking the salt on a full stomach. The calcium lactate gave a very similar curve except that when given on an empty stomach the plateau of the curve was much flatter. This is probably due to the fact that the hyperperistalsis induced by the lactates caused such a rapid movement of the material through the bowel that proper absorption could not take place. At this point it seems to be worth while

repeating the statement previously made as to maximal optimal doses. The 10 grams of calcium gluconate powder and the equimolecular amount of the lactate is not only the maximal dose from the standpoint of comfort in taking the substances but is also the largest amount that can be taken without having a *decrease* in the amount of calcium ion absorbed. This apparent paradox is readily explicable when we take into consideration the fact that overdosage sets up a violent diarrhea which causes such a rapid passage through the bowels of the salts that proper absorption can not take place.

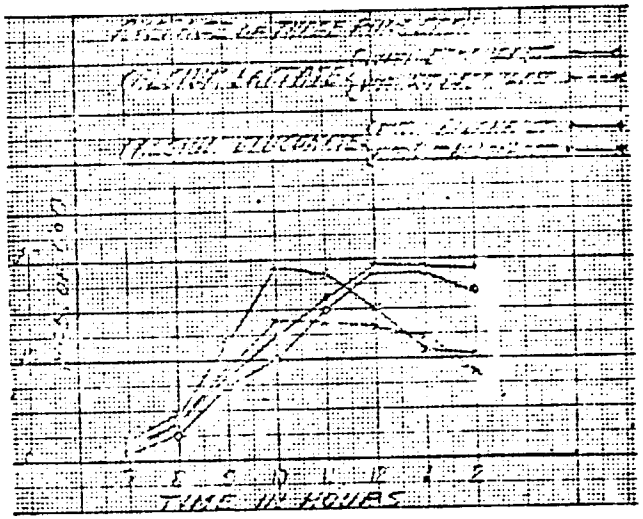

FIG. 3

CONCLUSIONS

1. Using urinary calcium as an index, the extent of calcium absorption following peroral administration of calcium gluconate and calcium lactate has been studied in three healthy individuals and calcium lactate has been studied in three healthy individuals.

2. There is a slight but very definite diuretic effect in this series.

3. There is a maximal dose beyond which the diarrheal effect begins to outweigh the size of the dose. The salt begins to pass through and out of the intestine too rapidly to be absorbed properly. The gluconate salt gives fewer subjective symptoms as compared with the lactate salt.

4. It is much better to administer the calcium salt after a meal. There is a smoother and greater absorption with a minimum of subjective distress. Also, for a given dose the physiological action of the ingested calcium persists over a more protracted period of time.

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I. P. PAVLOV HIGHER MEDICAL INSTITUTE OF PLOVDIV, BULGARIA
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CHANGES IN THE LIPOLYTIC ACTIVITY OF ARTERIAL WALL IN ALBINO RATS

II. Effect of magnesium gluconate

S. Markov, D. Mitkov and *H. Milenkov*

The authors report that the lipolytic activity (determined according to the modified method of Zemplenii and Grafnetter) of the aortal wall from albino rats (250—270 gr) is very increased ($P < 0{,}001$) towards the hyperlipemic substrate (animal fats), containing magnesium gluconate. This effect (18,8 eq) is greater than the effect of L-ascorbic acid (15.2 eq) and the non-saturated fatty acids (12 eq). They assume that the increased lipolytic activity under the influence of magnesium gluconate is due to the activation of ferment reaction by the magnesium ions.

According to data in literature, the disturbed matabolism of magnesium in the organism contributes to the development of atherosclerosis (9, 12). It is also known that the content of magnesium in blood serum in atherosclerosis incessantly is descreased in parallel with the development of the disease, and that magnesium helps the decrease of cholesterol level in blood (5).

Bearing in mind that, we decided to verify what is the effect of some magnesium compounds upon the lipolytic activity of arterial wall.

From the magnesium compounds we chose magnesium gluconate because we assume that the metabolic gluconic acid will secure the resorption of gluconate by every cell. On the other hand, the low degree of electrolytic dissociation of magnesium gluconate will allow Mg^{2+} not to be deposited in the intestines as magnesium phosphate, while the gluconic acid will help its resorption by the walls of the intestines.

The magnesium gluconate necessary for the experiments we obtained by adding to the water solution of calcium gluconate an equivalent quantity of magnesium sulphate on heating, and we filtered out the formed calcium sulphate.

Methods

For solving the above problem we subjected to experiment 17 albino rats, male, weighing 250—270 g. Te lipolytic activity of their aortal wall was determined according to the modified method of Zemplenii and Grafnetter (13). The aorta, after removing the adventitia, was broken to small pieces and 35—40 mg of its tissue was placed in test tube with 2 ml of hyperlipemic serum (with total lipids about 1,250 mg %). The latter was obtained from a dog, which 4 hours before that ate up 10 g/kg bodyweight butter + 100 mg/kg magnesium gluconate, injected intravenously, 5 minutes before the taking of blood. This mixture was incubated in water bath at 37° for 150 min, stirring continuosly. Parallel with the test and the control test tube was placed only with hyperlipemic serum. The lipolytic activity of arterial wall was determined according to the difference in the content of the free fatty acids (FFA) in the test and control test tube. We determined FFA according to Duncombe's metod, 1964 (8).

Results and discussion

As it is visible from the present table the lipolytic activity of the aortal wall is very increased towards the hyperlipemic substrate containing magnesium gluconate in comparison with the substrate non-containing gluconate.

Lipolytic activity of arterial wall (eq) I ml/1 g. Substrate — hyperlipemic serum with:

Butter — 10 g/kg		Butter — 10 g/kg + Magnesium gluconate — 100 mg/kg
8,1 ± 0,68 (11)	M ± m p < 0,001	18,8 ± 1,1 (6)

Note: — in brackets is shown the number of animals.

This gives us ground to accept that magnesium gluconate by means of the increased lipolysis in the arterial wall decreases „the atherogenic potential" of lipids, containing saturated fatty acids.

It is known, that β-lipoproteins, especially those (Sf. 10—20, 20—100), which are considered as pathogenic factor in atherosclerosis, because of their big and hydrophobe molecule, with difficulty pass through the arterial wall and most frequently are susceptible to be detained subendothelially. In the works of a series of authors (6, 10, 7) is pointed out, that from the intima towards the adventitia pass as less lipoproteins, as greater is their molecule. The liberation of β-lipoprotein molecule from triglycerides, makes easier its passing from the intima towards the media, impedes its deposition in the arterial wall. A basic part in this metabolism of lipids belongs to the lipolytic enzyme systems of arterial wall.

The decreased lipolytic activity of the aortal wall i. e. its decreased capacity for destructing the triglycerides appears to be an important prerequisite in the development of atherosclerosis (1).

The circumstance, that magnesium gluconate increases the lipolytic activity of the aortal wall towards one of the most widely used „atherogenic" foods, gives us ground to accept, that in decreasing the mo'ecule of -lipoproteins, by lipolysis of their triglyceride component, it would impede the development of lipoidosis and atherosclerosis.

By comparing the obtained results with these of other investigations of ours in this field it is visible, that magnesium gluconate enhances much more the lipolytic activity of the arterial wall (18,8 μ/eq) in comparison with ascorbic acid (15,2 μ/eq) (2) or with the non-saturated fatty acids (12 μ/eq) (3).

What is the intimate mechanism of activity of magnesium gluconate, whether by activation of the enzymes or by effect upon the substrate it is arrived to an increased lipolysis in the arterial wall is a question to which it is difficult to answer in a categorical way. It is known however, that the activity of the ferments in the cytoplasm and intracellular structures depends in a great degree on ion concentration and especially of K^{++}, Mg^{++}, HPO_4^{--} (11). And other authors point out, that such ions as Mg^{++}, Mn^{++} and Ca^{++} can play the role of activators of ferment reaction, changing the electrolytic structure of definite groups of the substrate and playing the part of connecting link with the proteins in the formation of ferment-substrate complexes (4).

With this activity of Mg^{++} ions we would explain and the higher lipolytic activity of the arterial wall in our experiments.

It would be impossible to presume in this case the presence of induced by the substrate synthesis of lipolytic enzymes at the level of the cell, since the time of incubation is very insufficient and at that in an experimental set carried out in vitro.

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ИЗМЕНЕНИЯ В ЛИПОЛИТИЧЕСКОЙ АКТИВНОСТИ СОСУДИСТОЙ СТЕНКИ У БЕЛЫХ КРЫС

II. Влияние глюконата магния

С. Марков, Д. Митков, Х. Миленков

Авторы сообщают, что липолитическая активность, стенки аорты белых крыс, определяемая видоизмененным методом Zemplenii и Grafnetter, (250—270 г) повышается сильно ($P < 0{,}001$) к гиперлипемическому субстрату (животные жиры), содержащему глюконат магния. Этот эффект (18,8 экв) больше эффекта L-аскорбиновой кислоты (15,2 экв) и ненасыщенных жирных кислот (12 экв). Авторы считают, что повышенная липолитическая активность под влиянием глюконата магния является следствием активирования ферментативных реакций ионами магния.

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THE TOXICITY AND RATE OF DISAPPEARANCE OF INTRACISTERNALLY INJECTED CALCIUM SALTS IN THE DOG

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In studying the effects of intracisternally administered electrolytes on the blood pressure of dogs it was observed that substances such as citrate, oxalate and phosphate, which diminished the ionized calcium of the cerebrospinal fluid, had a pressor action and caused muscular twitchings (1). Evidence that the ionized calcium of the cerebrospinal fluid is diminished in the uremic state (unpublished data) suggested the possibility that calcium salts introduced intracisternally might be of some therapeutic value in patients with uremia. Before resorting to such a procedure data were obtained concerning the toxic effects and rate of disappearance of various calcium salts when administered to dogs in this manner.

METHODS

Observations were made on 28 dogs in respect to the toxicity, and on 6 dogs in respect to the rate of disappearance of various doses of the lactate, gluconate and chloride of calcium injected intracisternally during morphine or sodium pentobarbital anesthesia.

In addition to solutions of these salts suspensions of calcium lactate were used with the idea that a more prolonged action might be obtained. In view of the antagonism between salts of calcium and magnesium when administered by other routes (2) it was thought that larger doses of calcium salts might possibly be tolerated intracisternally if small amounts of magnesium salts

TABLE 1*

The lethability of calcium salts when administered into the cisterna magna

DATE	WEIGHT OF DOG	CALCIUM SALT INJECTED	CONCENTRATION OF CALCIUM SALT USED	AMOUNT OF CALCIUM INJECTED	SURVIVED	NARCOTIC USED	SPINAL FLUID CONT.	
							Before calcium	21 to 43 hours after calcium
	kgm.		*mgm. per liter*	*mgm. per kgm.*				
February 25	8.7	Lactate	50 (solution)	0.23	Yes	Nembutal		
February 28	12.1	Lactate	50 (solution)	0.17	Yes	Nembutal		
February 25	10.2	Lactate	100 (solution)	0.40	No	Nembutal		
February 21	12.5	Lactate	200 (suspension)	0.64	Yes	Morphine	3	1290
February 21	5.2	Lactate	125 (suspension)	0.96	Yes	Morphine	10	8000
February 22	8.0	Lactate	100 (suspension)	0.50	Yes	Morphine	5	870
February 22	6.1	Lactate	62 (suspension)	0.40	Yes	Morphine	11	1890
February 22	4.8	Lactate	500 (suspension)	4.0	No	Morphine		
February 20	4.5	Lactate	100 (suspension)	8.8	No	Morphine		
February 21	14.3	Lactate	1000 (suspension)	0.55	No	Morphine		
February 21	6.2	Lactate	250 (suspension)	1.61	No	Morphine		
February 22	8.5	Lactate	90 (suspension)	0.41	Yes	Morphine	3	1810
February 23	11.3	Gluconate	100	0.35	Yes	Nembutal		
February 27	8.1	Gluconate	50	0.25	Yes	Nembutal		
March 1	10.2	Gluconate	50	0.20	Yes	Nembutal		
March 25	5.8	Gluconate	50	0.35	No ✓	Nembutal		
March 30	6.1	Gluconate	30	0.20	No ✓	Nembutal		
February 23	13.4	Gluconate	230	0.68	No ✓	Nembutal		
December 17	15.0	Chloride	100	0.27	Yes	Morphine	13	61
December 29		Chloride	100	0.27	Yes	Morphine	61	12

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December 31.....		Chloride	100	0.27	Yes	Morphine	12	13
January 3.....		Chloride	100	0.27	No (meningitis)	Morphine	13	2500
December 27.....	13.4	Chloride	50	0.15	Yes	Morphine		
January 7.....	12.5	Chloride	50	0.16	Yes	Morphine		
January 16.....		Chloride	50	0.16	Yes	Morphine		
February 5.....		Chloride	50	0.16	Yes	Morphine		
February 26.....	8.0	Chloride	50	0.25	Yes	Nembutal		
March 13.....	8.0	Chloride (also 1 cc. M/10 $MgCl_2$)	200	1.0	No	Morphine		
March 13.....	6.1	Chloride (also 1 cc. M/10 $MgCl_2$)	100	0.65	No	Morphine		
April 22.....	8.9	Chloride (also 1 cc. M/30 $MgCl_2$)	90	0.40	No	Nembutal		
April 22.....	6.8	Chloride (also 1 cc. M/40 $MgCl_2$)	84	0.50	No	Nembutal		
April 22.....	5.8	Chloride (also 1 cc. M/40 $MgCl_2$)	50	0.35	No	Nembutal		
April 22.....	5.5	Chloride (also 1 cc. M/60 $MgCl_2$)	30	0.20	Yes	Nembutal		

* This table includes only experiments in which calcium was injected before other electrolytes were administered. The lethal dose of calcium is greater than the values given in the table if calcium salts are injected after citrate, oxalate, phosphate or potassium administration.

TABLE 2

Rate of disappearance of intracisternally injected calcium salts from the cerebrospinal fluid

DOG 1		DOG 2		DOG 3		DOG 4		DOG 5		DOG 6		DOG 7	
Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium	Hours after injection	Cerebrospinal fluid calcium
1 cc. M/10 $CaCl_2$		1 cc. M/20 $CaCl_2$		1 cc. M/20 calcium gluconate		1 cc. M/20 calcium lactate		1 cc. M/20 calcium gluconate		1 cc. M/20 calcium gluconate		1 cc. M/20 calcium lactate	
	mgm. per cent		*mgm. per cent*		*mgm. per cent*		*mgm. per cent*		*mgm. per cent*		*mgm. per cent*		*mgm. per cent*
0.0	3.2	0.0	4.2	0.0	5.1	0.0	4.8	0.0	5.0	0.0	5.0	0.0	4.9
0.1	71.4	0.2	28.2	0.2	38.6	0.2	62.4	0.2	51.2	0.2	58.2	0.2	23.4
1.5	11.9	1.0	8.3	1.0	18.4	1.0	5.8	1.0	10.3	1.2	20.6	1.0	5.1
2.5	9.5	2.0	6.1	2.0	11.0	2.0	6.6	2.0	11.5	2.0	10.9	2.0	3.5
3.0	9.3	2.3	5.4	2.7	8.3	3.0	5.7	3.0	7.9	3.0	9.0	2.5	5.4
3.8	5.9	1 cc. M/20 calcium lactate		3.5	6.5	4.0	6.2	3.4	5.0	4.0	4.3	1 cc. M/20 calcium gluconate	
4.0	7.2	0.0	5.4	1 cc. M/20 calcium lactate		5.0	6.4	1 cc. M/20 calcium lactate		5.0	5.6	0.0	5.4
1 cc. M/4 calcium lactate suspension		0.3	30.8	0.0	6.5	6.2	5.5	0.0	5.0	6.0	5.6	1.0	9.8
0.0	7.2	1.0	17.2	0.2	53.4	1 cc. M/20 calcium chloride		0.2	19.2			2.0	5.2
0.2	125.0	2.0	9.8	1.0	10.5	0.0	5.5	1.0	7.1			3.0	4.8
1.0	34.3	3.0	7.3	2.0	7.4	0.2	39.6	2.0	7.3			3.2	4.7
2.0	13.2	3.8	5.7	2.2	6.4	1.0	3.8	3.4	3.4				
3.0	8.3	1 cc. M/20 calcium gluconate		1 cc. M/20 calcium gluconate		2.2	5.1						
3.2	5.3	0.0	5.7	0.0	6.4	3.2	5.3						
3.4	5.4	0.2	79.7	0.2	52.9								
3.9	5.8	1.0	19.6	1.0	8.8								
4.1	5.7	2.0	9.2	1.3	5.4								
		3.0	7.4										
		4.0	7.0										
		5.0	7.0										
		5.9	6.2										

were given at the same time and some experiments were performed to test this point.

The methods of sampling and analyses used were those described in a previous paper (1). It should be pointed out that there had been no intracisternal injections of other electrolytes in these dogs prior to the injection of the calcium salts.

RESULTS

The results of observations on the toxicity of calcium salts introduced intracisternally are summarized in table 1. In table 2 the data on the rate of disappearance of calcium salts from the cisternal fluid are presented.

DISCUSSION

Different dogs displayed considerable variation in their susceptibility to toxic effects; however, no marked difference was observed between solutions of the three salts studied. Doses of solutions of these salts containing 0.25 mgm. or less of calcium per kilogram of body weight were not fatal in nine out of ten experiments. Doses of 0.40 mgm. or more per kilogram of body weight were fatal in each of six observations, death occurring from respiratory paralysis. Intermediate doses were fatal in some instances and not in others. Inclusion of magnesium chloride with calcium chloride did not seem to increase the tolerance of the animals for calcium; neither did it seem to diminish the chances for survival. Meltzer (3) states that the respiratory depression resulting from intraspinally injected magnesium sulfate cannot be overcome by the intravenous injection of calcium chloride. On the other hand magnesium coma attended by a high serum magnesium level can be abolished by intravenous calcium chloride (2, 4).

Suspensions of calcium lactate were found to be considerably less toxic than solutions of calcium chloride and calcium gluconate of similar calcium content in respect to their immediate effects. However, the suspensions caused considerable irritation, as shown by the number of cells appearing in the cerebrospinal fluid

In every instance in which lethal doses of calcium salts were introduced into the cisterna, death was due to respiratory failure. The breathing first became shallow, then slow, diminishing to occasional gasps and death resulted in five to sixty minutes, occurring most rapidly with the larger doses. It had already been observed with other electrolytes that fatal doses acted by producing respiratory depression irrespective of whether or not there had been a preliminary stimulation of breathing (1). Several of the dogs recovered after their respiratory rates had been reduced to as low as three per minute. This respiratory inhibition by intracisternally introduced calcium salts is surprising in view of the fact that intravenous injection of calcium salts will relieve respiratory failure produced by previous intravenous administration of magnesium salts (2).

The effect of repeated intracisternal injections of calcium chloride were studied in two dogs. One of these died of bacterial meningitis after the fourth injection, but neither showed any evidence of a toxic cumulative action of calcium.

In studying the disappearance rates of intracisternally injected calcium chloride, calcium lactate, and calcium gluconate, each animal received two or more injections; that is, after the cerebrospinal fluid calcium value had returned to the neighborhood of its original level following the initial injection, another injection of a different calcium salt solution was made. In this manner two or more series of observations were made on each dog. Of the calcium salt solution, 1 cc. was injected in each case, and the samples of cerebrospinal fluid withdrawn for analysis were about the same volume. In most cases the solutions were either 0.1 M or 0.05 M. The difficulties of proper mixing and the continued withdrawal of fluid from the cisternal space probably account for many of the irregularities in the results; however, there seemed to be little difference in the behavior of calcium chloride, calcium lactate and calcium gluconate. Somewhat less respiratory depression was observed when the latter salt was injected. With all three salts the fall in concentration in the cerebrospinal fluid was very rapid during the first hour. In some instances normal values

elevated values were found after three or four hours. From the standpoint of solability and reaction calcium gluconate is preferable to calcium lactate and calcium chloride as a means of raising the calcium concentration in the cerebrospinal fluid.

SUMMARY

Solutions of calcium chloride, calcium gluconate and calcium lactate containing 0.25 mgm. or less of calcium per kilogram of body weight may be injected intracisternally in dogs without the occurrence of pronounced toxic reactions. Larger doses cause pronounced respiratory depression and 0.40 mgm. of calcium per kilogram of body weight was found to be a lethal dose in all instances. The simultaneous injection of magnesium chloride and calcium chloride did not increase the tolerance of dogs to calcium chloride.

No pronounced differences were found in the rate of disappearance from the cerebrospinal fluid of intracisternally injected calcium chloride, calcium gluconate and calcium lactate, although in some instances the calcium level remained considerably elevated for several hours.

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EARLY RESEARCH ON MAGNESIUM GLUCONATE.

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Pavia Section - Session of February 12, 1932.

The great importance which studies of vegetal and animal physiology during the past few years have imparted to magnesium, whose presence in the living organism appears specifically bound to fundamental biological functions has been, as is known, usefully increased by the extension of investigations on the pharmacological actions of the metal. This has resulted in a better knowledge of its action on the central and peripheral nervous system, on biliar scretion, on the contractions of the smooth muscular fibers, on the coagulation of the blood, etc.

On the other hand the use of magnesium, already affirmed in America for regional anesthesia, has found especially in France, through the work of Delbet, the most vocal recommendation for the treatment of various morbid conditions: diseases of the skin, Parkinson disease, infective, anaphylactic, precanderous, presenile, etc. conditions. In all these lesser known conditions there seems to appear a development of various magnesium preparations open to the possible choice of the physician. Nowadays oral magnesium treatments are generally given only in the form of magnesium chloride and magnesium sulfate. For the first salt, though widely used with good reason as bland purgatives, one cannot fail to note its rather irritating action even at small doses; for the second salt, the disgusting taste, the purgative action and the questioned absorption of magnesium are well known. Via hypodermic route, which would appear the choice method for many pathological conditions apt to benefit from magnesium, the chloride form is rather painful and irritating and in fact it is not used; the sulfate and hyposulfate forms are used, but in reality only for episodic treatments.

For these reasons we deem it useful to study a new magnesium salt which

would be readily soluble, injectable, suitable for prolonged treatments, and in any event such as to increase the number of products available to the physician. We turn our attention to organic magnesium gluconate, of atoxic anion, not foreign to the organism and which has already given good results in combination with Ca. Stoechiometric calculation axcribes 5.86% of Mg in gluconate. The product we use was obtained by oxidation of a glucose solution with bromine, elimination of the excess halogen, precipitation of the HBr formed with lead carbonate and silver oxide. The excess Pb and Ag was precipitated with sulfuric acid, the sulfuric acid was driven off and the solution which now contained only gluconic acid was treated with magnesium carbonate. The carbon dioxide was eliminated and magnesium gluconate was obtained, which was purified by successive crystallizations. The salt, from which it is very difficult to eliminate small quantities of water, appears as a very white powder, non-hygroscopic, readily soluble in water. One gram dissolves easily in 6 cc of water at 25°. The aqueous solution is slightly acid: pH = 5.8. The 10% aqueous solution in neutral glass withstands sterilization for 45 minutes under steam without damage: one notes merely a slight increase in the pH value (from pH 5.8 to 6.0). The solutions remain clear and colorless and are long lasting.

The following determinations were made: a) tolerance, b) toxicity, c) absorption, d) antianaphylactic capacity.

a) In animals and in man an endodermic, hypodermic, perivasal or endomuscular injection of 10% solution produces no pain or irritation. Subcutaneous injections of 10cc of 10% solution (Mg: g. 0.0586) are prefectly well tolerated without local or general manifestations.

"Per os" magnesium gluconate is tasteless and well tolerated, even at high doses. At excessive doses it produces a purgative action: in the rat - 20g/kg; in the guinea pig - 10 g/kg; in the rabbit - 5 g/kg. This action is

not observed in hypodermic or endovenous injections.

b) Comparative toxicity tests with Magnesium Sulfate were performed with rats, guinea pigs and rabbits, using equimolar solutions of the two salts. The table indicates the certainly lethal doses.

Animals	Administration (route	Magnesium gluconate x kg	$MgSO_4.7H_2O$ x kg	Concentration of solution	Number of experiments
rabbit	endovenous	cc 7-8	cc 7-8	g°/oo	0.2354	29
rat	subcutaneous	cc 40-45	cc 40-45	"	0.2354	33
guinea pig	endoperitoneal	cc 40-45	cc 40-45	"	0.2354	23

Therefore no differences in toxicity appear between magnesium gluconate and magnesium sulfate.

c) When magnesium gluconate is administered "per os", absorption proves rapid. Approximately one third of the magnesium administered is recovered in the urine of the first 24 hours. By subcutaneous injections the animals undergo prolonged narcosis, which appears promptly. Injections of g 3 per kg. in the rabbit produce typical magnesium narcosis, which however can be easily reversed by endovenous injection of Cl_2Ca. For greater doses the typical pattern of magnesium poisoning appears in a few minutes, accompanied by the usual phenomena: myosis, esophthalmy, paresis, tetraplegia, dyspnea, death.

d) Many careful tests were performed in order to seek, through gluconate, the antianaphylactic action attributed to magnesium.

Subcutaneous, endoperitoneal and endovenous injections of high quantities of the salt before the triggering injection in sensitized guinea pigs did not generally avoid the shock which occurred according to the usual pattern. Therefore an antianaphylactic action was not observed.

Translated by Carl Demrick Associates, Inc./ARB/db

PRIME RICERCHE SUL GLUCONATO DI MAGNESIO.

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Sezione di Pavia. - Seduta del 12 febbraio 1932.

Il grande rilievo che gli studi di fisiologia vegetale ed animale hanno in questi ultimi anni conferito al magnesio, la cui presenza nell'organismo vivente appare specificamente legata a fondamentali funzioni biologiche, è stato, come si sa, utilmente accresciuto dall'estendersi delle indagini sulle azioni farmacologiche del metallo. Ne è risultata meglio conosciuta l'azione sul sistema nervoso centrale e periferico, sulla secrezione biliare, sulla contrattilità delle fibre muscolari liscie, sulla coagulabilità del sangue, ecc.

D'altra parte l'impiego del magnesio, già affermatosi in America per anestesie regionali, ha trovato, specialmente in Francia per opera del Delbet, la più clamorosa raccomandazione pel trattamento di svariati stati morbosi: malattie della pelle, parkinsonismo, stati infettivi, anafilattici, precancerosi, presenili, ecc. In tutto questo movimento meno progredito appare forse l'apprestamento di svariati preparati di magnesio su cui possa svolgersi più adeguata la scelta del medico. Oggidì un trattamento magnesiaco per bocca non viene generalmente realizzato che con cloruro di magnesio e con solfato di magnesio. Al primo sale, largamente e con ragione adoperato, non si può tuttavia evitare l'appunto della notevole deliquescenza oltre al fatto di riuscire, a dosi di poco elevate, piuttosto irritante, ond'è accolto fra i blandi purganti; del secondo sale è troppo noto il sapore disgu-

stoso, il contestato assorbimento del magnesio, l'azione purgativa. Per via ipodermica, che apparirebbe di scelta per molti stati patologici che si beneficerebbero dal magnesio, il cloruro riesce piuttosto doloroso e irritante e non viene infatti adoperato: adoperato è il solfato ed anche l'iposolfito, ma in realtà solo per trattamenti episodici.

Per questi motivi stimammo di qualche utilità studiare un nuovo sale di magnesio che potesse riuscire bene solubile, iniettabile, idoneo a trattamenti prolungati ed in ogni caso tale da accrescere i prodotti a disposizione del medico. Volgemmo l'attenzione al gluconato di magnesio, dall'anione atossico, organico, non estraneo all'organismo e che buona prova ha già dato in combinazione col Ca. Il calcolo stechiometrico assegna al gluconato il 5,86 % di Mg. Il prodotto che adoperammo era ottenuto ossidando una soluzione di glucosio con bromo, eliminando l'eccesso dell'alogeno, precipitando l'HBr formatosi con carbonato di Pb e con ossido di Ag. L'eccesso di Pb e di Ag veniva precipitato con acido solfidrico, si scacciava l'acido solfidrico e la soluzione che conteneva ora soltanto acido gluconico ed impurezze veniva trattata con carbonato di Mg. Si eliminava l'anidride carbonica e si otteneva gluconato di magnesio che si purificava con successive cristallizzazioni. Il sale, nel quale è molto difficile eliminare completamente piccole quantità di acqua, appare come polvere bianchissima, non igroscopica, facilmente solubile in acqua. Un grammo sciogliesi facilmente in cc 6 di acqua a 25°. La soluzione acquosa è lievemente acida: pH = 5,8. La soluzione acquosa al 10 % in vetro neutro sopporta senza danno la sterilizzazione per 45 minuti a vapore fluente: si rileva soltanto un leggerissimo elevarsi del valore di pH (da pH = 5,8 a 6,0). Le soluzioni restano limpide ed incolori, durevolmente.

Vennero compiuti accertamenti: a) di tolleranza, b) di tossicità, c) di assorbimento, d) di capacità antianafilattica.

a) Negli animali e nell'uomo l'iniezione endermica, ipodermica, perivasale, endomuscolare di sol. 10 % non provoca dolori o fatti irritatori. Iniezioni sottocutanee di cc 10 di sol. 10 % (Mg: g 0,0586) risultano perfettamente tollerate senza manifestazioni locali o generali.

Per *os* il gluconato di magnesio risulta *insapore*, bene tollerato anche a dosi elevate. A dosi eccessive provoca azione purgativa: ratto g 20 per kg, cavia g 10 per kg, coniglio g 5 per kg. Questa azione non si rileva per iniezioni ipodermiche nè endovenose.

b) Saggi comparativi di tossicità con solfato di Mg furono

compiuti in ratti, cavie, conigli mediante soluzioni equimolecolari dei due sali. La tabellina riporta le dosi sicuramente mortali.

	A	B	C	D	E	F
	Animali	Via sommin.	Gluconato Mg × Kg	$MgSO_4.7H_2$ × Kg	Concentr. della soluz.	Numero delle esperienze
a	coniglio	endovenosa	cc 7-8	cc 7-8	g‰ 0,2354	29
b	ratti	sottocutan.	» 40-45	» 40-45	» » »	33
c	cavie	endoperiton.	» 40-45	» 40-45	» » »	23

Non risultano quindi differenze di tossicità fra gluconato e solfato di magnesio.

c) Somministrando *per os* gluconato di Mg l'assorbimento si rivela pronto. Un terzo circa del Mg somministrato si ritrova nell'urina delle prime 24 ore. Per iniezioni sottocutanee si ha negli animali narcosi prolungata, di pronta insorgenza. Iniezioni di g 3 per kg nel coniglio provocano la tipica narcosi da Mg, facilmente redimibile dalla iniezione endovenosa di Cl_2Ca. Per dosi superiori il quadro tipico dell'avvelenamento da Mg si rivela in pochi minuti con la consueta fenomenologia: miosi, esoftalmo, paresi, tetraplegia, dispnea, morte.

d) Accurate e numerose prove furono praticate per ricercare mediante il gluconato l'azione antianafilattica attribuita al Mg.

Iniezioni sottocutanee, endoperitoneali, endovenose di quantità elevate del sale prima dell'iniezione scatenante in cavie sensibilizzate non evitarono in linea generale lo *shock* che si svolse col consueto quadro. Un'azione antianafilattica non fu quindi rilevata.

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THE EFFECT OF MAGNESIUM GLUCONATE ON EXPERIMENTAL ATHEROSCLEROSIS IN PIGEONS, AND ON THE CONTENT OF SULFHYDRYL AND DISULFIDE GROUPS IN AORTA AND LIVER

D. Staneva-Stoyčeva, S. Markov, M. Zlateva and *G. Antov*

The prophylactic activity of magnesium gluconate, at a daily dose of 100 mg/kg bodyweight orally, has been studied in pigeons with experimental cholesterol atherosclerosis. The authors established a protective effect of magnesium gluconate, expressed by a slighter increase of cholesterol (with 28%) of β-lipoproteins (with 22%) in serum, and by slighter morphologic (macroscopic and histological) changes in the aorta and liver of the pigeons treated. They have confirmed their previous investigations establishing a decrease of sulfhydryl (—SH) groups and an increase of the disulfide ones (S—S) in the aorta and liver of the pigeons with atherosclerosis. In the pigeons treated with magnesium gluconate, this increase of disulfide groups is statistically less reliable in comparison with the pigeons having obtained only atherogenetic diet.

In the literature there exist fragmentary and contradictory data with egard to the connection between magnesium content in the organism and he disturbed lipid metabolism in atherocslerosis. It has been pointed out hat the disturbed metabolism of magnesium in the organism favours the evelopment of atherosclerosis. Bersohn and Oelofse (1957) established an bvious regressive correlation between the level of serum magnesium and the evel of cholesterol. The experimental investigations by Vitale and al. (1957) owed that the artificial decrease of serum magnesium in rats increases the egree of hypercholesterolemia provoked through feeding on a diet containing holesterol and cholic acid, while an eight-time increase of the amount of agnesium in food decreases or retards the deposition of lipids in the aorta id atrioventricular valves. At that, the authors have not established a decrese f serum level of cholesterol, which makes them assume that probably magesium has some local protective activity on the vessel wall. Hegsted and al. 957) demonstrate also a close correlation between the degree of experiental cholesterol atherosclerosis and the level of serum magnesium. The clinical bservations by Malkiel—Shapiro and al. (1956) speak about a favourable cli-

nical effect upon patients having contracted cardiac infarction, by means of magnesium sulfate applied parenterally,—and about the normalization of the abnormal serum level of lipoproteins. Other authors, however (D. F. Brown and al., 1958) have not established in their investigations on healthy persons and patients with recent myocardial infarction a direct dependence between serum magnesium and the level of lipids. Nikamura and al. (1965) have established that the degree of lipid deposition in the aorta of rabbits fed on cholesterol, may markedly become increased through feeding on a diet poor in magnesium, but it does not decrease through feeding on fodder rich in magnesium.

Taking into consideration those data and the enormous significance of magnesium ion for many vitally important metabolic and enzymatic processes in the organism, we undertook the task of investigating the effect of magnesium gluconate on some indexes of lipid metabolism and on the degree of the atherosclerotic process in the experimental atherosclerosis of pigeons. Basing ourselves upon our previous investigations demonstrating an existing regressive correlation between the content of SH—groups and the level of serum lipids, we have followed up, moreover, the effect of magnesium gluconate on the content of sulfhydryl and disulfide groups in aorta and liver. We have found the reason for choosing magnesium gluconate as magnesium compound in the circumstance that the metabolic gluconic acid might allow a better and more complete assimilation of magnesium by every cell, and that the lower degree of electrolytic dissociation of magnesium gluconate would not allow its deposition in the gastrointestinal tract as magnesium phosphate, while the gluconic acid would ensure its better resorption.

Material and methods

We implemented the experiments on 30 pigeons of both sexes, distributed equally into two groups, with a weight of 260—400 g. We provoked experimental atherosclerosis by daily feeding the pigeons in the course of 12 weeks on a special atherogenetic diet containing cholesterol (2 g/kg bodyweight), sunflower-seed oil (5 g/kg bodyweight) and wheat flour necessary for making a dough, from which we used to give at a rate of 1 g/100 g bodyweight. The pigeons of the first group had only atherogenetic food completed with oats and water at will. The pigeons of the second group had the same atherogenetic diet to which magnesium gluconate at a dose of 100 mg/kg bodyweight was admixed. We obtained the magnesium gluconate necessary for the experiment by adding an equivalent quantity of magnesium sulfate on heating to a 10% water solution of calcium gluconate, and we filtered out the formed calcium sulfate.

Pigeons' bodyweight was followed up periodically. The pigeons were killed in the end of the 3rd month. Just before killing, blood was taken and we investigated the content of cholesterol (according to Homalka) and of β-lipoproteins (according to M. Burstein and J. Samaille). Microscopic assay of aorta and liver was made, and material from liver and the initial part of aorta was taken for histological investigation. We determined, in homoge-

nates of aorta and liver, the content of sulfhydryl groups according to the method of Kolthoff and Harris, and of the disulfide ones according to Okulov's modification of Karter's method. Paraffin slices, 6 μ wide, were stained with hemalaun-eosin, toluidine blue of Ph 4.0, and according to Van Gieson's method. Frozen slices from aorta and liver were stained with Sudan III and Sudan black for lipids.

Results

The results of investigation of the content of cholesterol and β-lipoproteins in the blood of pigeons of the first and second groups are represented in table 1.

Table 1

Group	Cholesterol (mg %)	β-Lipoproteins (U)
I (only cholesterol)	1850.8±142	230.3±26.7
II (Cholesterol and magnesium gluconate)	1331.2±101 (— 28%) P<0.01	178.6±32.5 (— 22%) P>0.05

Table 2

μ Mg	Organ	Control	St. r. r.	I group	St. r. r.	II group	Sr. r. r.	M/I and II r.
	Aorta	5.2738±0.130	—	3.3817±0.232	0.001	4.4555±0.179	0.002	0.001
	Liver	31.5974±0.392	—	26.6468±0.654	0.001	28.6828±0.482	0.001	0.025
	Aorta	4.327±0.247	—	5.7454±0.350	0.01	5.0015±0.194	0.025	non investig.
	Liver	21.120±0.492	—	25.6178±0.592	0.001	23.6324±0.398	0.001	0.01

As it is visible from the present table, as a result of the daily feeding of the pigeons, in the course of 12 weeks, on an atherogenic diet, there appears a marked increase of the level of cholesterol in the blood (normally 433 mg %) and of β-lipoproteins (normaly 41 U).

In the pigeons treated with cholesterol and magnesium gluconate, the amount of cholesterol is by 28% lower in comparison with those treated only with cholesterol, the difference being statistically reliable at a $P<0.01$. The amount of β-lipoproteins in the same pigeons is by 22% lower in comparison with those treated only with cholesterol, but, because of the great individual deviations, the difference is not statistically reliable.

The results of the investigation of the content of sulfhydryl and disulfide groups in the homogenate from liver, aorta of the control healthy pigeons, — of those treated only with cholesterol (I group), and of those treated with cholesterol and magnesium gluconate (II group) are represented in table 2.

As it is visible from the table, also in this experiment was confirmed the established, in our previous investigations, marked and statistically reliable decrease of the content of the sulfhydryl groups in the liver and aorta of the pigeons on an atherogenic diet. The content of disulfide groups in the same organs is statistically reliable higher in comparison with the control pigeons. We established a similar decrease of sulfhydryl groups and an increase of the disulfide ones — although not so much obviously — also in the pigeons on an atherogenic diet, and treated with magnesium gluconate. The difference in the content of SH groups in the liver and aorta of the pigeons in the Ist and IInd groups is statistically reliable at $P < 0.025$ and 0.001.

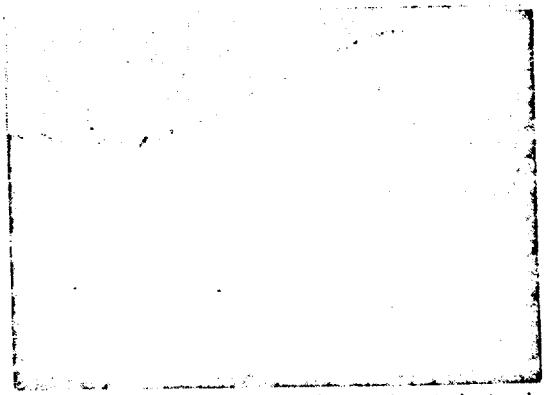

Fig. 1. Pigeon treated only with cholesterol. Deposition of an abundant quantity of lipids in the intima and focally in the internal part of the media. Accumulation of acid mucopolysaccharides in the media

The difference is statistically reliable also in the content of disulfide groups in the liver of the pigeons in the Ist and IInd groups, while this one for the content of the same groups in the aorta is not reliable. The macroscopic assay of the aortas taken from the pigeons in the Ist group (treated with cholesterol) has shown a presence of a greater or smaller amount of tiny lipid spots on the intima, and being of the range of a millet seed in a half out of them. In the others, the intima is almost plain, or only single spots and a hardly noticeable roughness without any change of colour are to be noted. Only in one pigeon (№ 13) big, formed patches of the type of the atherosclerotic ones in man were found. The livers (excepting № 14) are submitted to a severe fatty dystrophy.

In the pigeons №№ 21, 22, 23, 25, 27, 28, 29 and in the IInd experimental group, we have observed some thickening, roughness and paleness of the intima of aorta. Single and hardly noticeable tiny lipid spots were to be seen in the other pigeons. The livers of the pigeons without any lipid spots on the aortal intima have shown only data for cyanosis, while those, in which there were lipid spots, were submitted to a moderate fatty dystrophy.

The histological investigation of the aortas of the pigeons in the Ist group showed an almost diffuse extra- and intracellular deposition of small dispersed lipids in the intima and on the internal elastic membrane with a penetration, in some limited places, also into the internal part of the media (fig. 1). Very few lipid depositions in the intercellular substance were to be demonstrated in the media itself. There was established an increase of the quantity of acid mucopolysaccharides, which was irregular and was mostly in the internal part of the media around the places where lipids were deposited. The collagenous fibers in these sections have lost their staining pro-

perties and aspect, as if they had been melt into the greater quantity of the basic substance.

In the livers, there was observed an abundant deposition of lipids in the hepatic and Kupffer cells, the lipid substances occupying the greater part of their cytoplasm.

The investigations on the aortas of the pigeons in the IInd group have shown a slight folding of their internal part towards the lumen, muscular cells, elastic fibers, many collagenous fibers and basic substance participating in the bulging areas. On the whole, an increase of the collagenous connective tissue in the intima was to be noted (fig. 2). Lipid accumulations were found only in some single places in the intima of some pigeons (figures 3 and 4). In the media, lipids were found only in some single muscular cells of some pigeons investigated (fig. 5).

Discussion of the results

As it is visible from the given results that magnesium gluconate preventively applied from the very beginning of having the pigeons on an atherogenic diet, has some protective effect with regard to the development of experimental atherosclerosis, and it is expressed both in the slighter increase of cholesterol and β-lipoproteins in serum, and in the slighter morphological — microscopic and histological — changes in the aortas and livers of the treated pigeons. In keeping with these changes is also the slighter decrease, established by us, of the content of sulfhydryl groups, and the slighter increase of the quantity of disulfide groups in the aortas and livers of the pigeons treated with magnesium gluconate in comparison with those treated only with cholesterol. We have established in a series of our experimental investigations (C. Stoičev and D. Staneva-Stoičeva, 1965; D. Staneva-Stoičéva, C. Stoičev and M. Zlateva, 1965; C. Stoičev, D. Staneva-Stoičeva, 1966; D. Staneva-Stoičeva, T. Stoičev and M. Zlateva), and in our clinical investigations (M. Zlateva, G. Antov 1967; M. Zlateva, G. Antov, 1967; M. Zlateva, G. Antov, M. Zlateva, G. Antov, 1967), a similar regressive correlation between the content of sulfhydryl and disulfide groups in the serum, aorta and livers, and the biochemical and morphological changes in atherosclerosis. As to the mechanism through which the magnesium gluconate exerts its protective antiatherosclerotic effect, our investigations so far have not allowed us to precise it. Only some assumptions may be made. The important role of magnesium for the function of a series of enzymic systems in the organism is well known. It is pointed out, for instance, that one of the effects of magnesium deficiency is the uncoupling of the oxidizing phosphorylation of mitochondrias, and the latter have a close relation to the metabolism of fatty acids (Vitale and al., 1957). Klein and Johnson (1954) and Tulpule and William (1955) established, in case of magnesium deficiency, an uncoupling of the oxidizing phosphorylation accompanied by a deficiency of essential fatty acids, the role of the latter in the process of atherogenesis having been well known, thereat. Moreover, it is known that the atherosclerotic process is accompanied by a disturbance of the activity of a series of oxidizing and lipolytic enzymes (Adams and al., 1962, 1963; M. Sandler and G. Boune, 1960) a greater

Fig. 2. Pigeon treated with cholesterol and magnesium gluconate. Fibrosis of the intima. Hemalaun-eosin staining

Fig. 3. Pigeon treated with cholesterol and magnesium gluconate. Deposition of lipids in the intima. Sudan black staining

Fig. 4. Pigeon treated with cholesterol and magnesium gluconate. Single lipophages in the intima. Hemalaun-eosin staining

Fig. 5. Focal deposition of lipids in the media. Sudan black staining

part of which are thiol enzymes. It is possible that the increase — provoked by magnesium gluconate — of the decreased content of SH— groups might condition the improvement of the oxidizing and lipolytic processes, and, thereby, it might lead to a favourable influence upon the atherosclerotic changes in the pigeons. It is possible, after all, that the metabolism of acid mucopolysaccharides in the blood vessel wall is influenced, since a great importance is given to their disturbance in the pathogenesis of atherosclerosis. In this respect, are of interest the data by T. Lauson and al. (1966) establishing that magnesium ethylenediaminetetraacetate, applied parenterally in rabbits with experimental atherosclerosis, leads to removing the atherosclerotic plaques and to restoring the normal content of chondroitinsulfate and neutral mucopolysaccharides.

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ВЛИЯНИЕ ГЛЮКОНАТА МАГНИЯ НА ЭКСПЕРИМЕНТАЛЬНЫЙ АТЕРОСКЛЕРОЗ У ГОЛУБЕЙ И НА СОДЕРЖАНИЕ СУЛЬФГИДРИЛЬНЫХ И ДИСУЛЬФИДНЫХ ГРУПП В АОРТЕ И ПЕЧЕНИ

Д. Станева-Стойчева, С. Марков, М. Златева, Г. Антов

Исследуется профилактическое действие глюконата магния в дозе 100 мг/кг в день per os на голубях с экспериментальным атеросклерозом, вызванным холестеролом. Отмечается протективный эффект глюконата магния, выраженный в более слабом повышении холестерола (на 28%), липопротеинов (на 22%) в сыворотке и более слабых морфологических (макроскопических и гистологических) изменениях в аорте и печени у получивших глюконат магния голубей. Подтверждается установленное при других исследованиях авторов понижение сульфгидрильных групп (—SH) и увеличение дисульфидных (S—S) групп в аорте и печени голубей, заболевших атеросклерозом. У голубей, которым применили глюконат магния, это понижение сульфгидрильных групп и увеличение дисульфидных групп статистически достоверно в меньшей степени, чем у голубей, бывших только на атерогенной диете.

Animal and Human Studies on Ferrous Fumarate, an Oral Hematinic

By M. C. Berenbaum, K. J. Child, B. Davis, Helen M. Sharpe and E. G. Tomich

THE ORAL TREATMENT of hypochromic anemia with inorganic salts of iron occasionally results in gastrointestinal distress, and massive doses produce necrosis of the gastric mucosa and liver (Forbes, 1947; Smith, 1952; Swift, Cefalu and Rubell, 1952; Luongo and Bjornson, 1954). A recent survey (Hoppe, Marcelli and Tainter, 1955) recorded 23 deaths following accidental or deliberate poisoning with ferrous sulphate over 100 years; a further 36 cases required hospitalization.

Attempts to find less toxic forms of iron for oral therapy have led to the introduction of the gluconate and succinate, and more recently the fumarate.

The animal experiments described below were designed to compare the toxicologic and hematinic properties of ferrous fumarate with those of the sulphate, gluconate and succinate. The hematologic studies conducted on patients with hypochromic anemia were concerned with ferrous fumarate only.

Animal Studies

Acute Oral Toxicity in Mice

The substances compared in these tests were ferrous fumarate, ferrous sulphate A.R., ferrous succinate and ferrous gluconate B.P.C. The ferrous iron contents of these four salts are 33.0, 20.0, 24.8 and 11.7 per cent, respectively. The sulphate was administered as an aqueous solution and the other three compounds as aqueous suspensions containing 0.1 per cent w/v of tragacanth. Groups of 10 male fawn mice (GFF strain, bodyweights 17 to 22 Gm.) were dosed orally and then observed for seven days, when the percentage mortalities were recorded. The LD_{50} values, which were calculated according to de Beer (1945) and expressed in mg. Fe/Kg., were fumarate 630, succinate 560, gluconate 320 and sulphate 230. Thus the relative toxicities were fumarate 1, succinate 1.1, gluconate 2.0 and sulphate 2.7.

The oral LD_{50} value for ferrous fumarate administered to male albino rats (WAG strain, bodyweights 100 to 150 Gm.) was 580 mg. Fe/Kg.

Subacute Oral Toxicity in Rats

The subacute oral toxicities of the four ferrous salts were compared in albino rats of the WAG strain.

Ferrous sulphate solution and suspensions of the fumarate, gluconate and succinate containing 20 mg. Fe/ml. were employed in this experiment.

Forty-five male and 45 female rats (40 to 100 Gm. bodyweight) were randomly distributed into nine groups of five males and five females. One group was not dosed and served as controls, while the other eight groups received oral doses of one or other of the iron compounds at a level of 50 or 100 mg. Fe/Kg. The animals were individually weighed at intervals and dosed daily, excluding weekends. After 12 weeks' dosing, the

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Table 1.—*The Effects of 4 Iron Compounds on Growth Rate in Rats*

Daily oral dose (mg. Fe/Kg.)	Group mean increases in body weight ± S.E. (Gm.) after 12 weeks' dosing									
	Controls		Fumarate		Sulphate		Gluconate		Succinate	
	male	female	male	female	male	female	male	female	male	female
0	131 ± 13.6	92 ± 6.6								
50			156 ± 11.7	101 ± 5.8	129 ± 10.7	73 ± 11.7	172 ± 10.2	106 ± 11.1	145 ± 6.8	95 ± 8.3
100			136 ± 12.8	87 ± 10.7	113 ± 10.8	84 ± 8.8	136 ± 13.3	85 ± 7.5	135 ± 11.1	96 ± 12.4

Table 2.—*The Emetic Effects of 4 Iron Compounds in Cats*

Cat No.	Fumarate				Sulphate			Gluconate			Succinate		
	Oral dose in mg. Fe/Kg.												
	40	45	60	80	10	15	20	20	40	15	20	30	40
1	−†	−	−	+	−	+	−	+	+	−	−	+	+
	−			+	−		−	−	+				
2	−		+‡	+	−	−	+	−	+		+		+
	−			−									
3	−		−	+	−	−	+	−	−		−		+
	−			−									
4		−			−		+	−	+	+		+	
5		−			−		+	−	+	−		+	
Number of cats vomiting / Number dosed	0/6	0/3	1/3	4/6	0/6	1/3	4/6	1/6	5/6	1/3	1/3	3/3	3/3
% response	0	0	33	67	0	33	67	17	83	33	33	100	100
AED_{50}* (mg. Fe/Kg.)			69			17			25		21		

*AED_{50} = approximate dose producing emesis in 50 per cent of cats dosed.
†— No vomiting.
‡+ Vomited.

red and total white cell counts and hemoglobin concentrations were determined on two males and two females from each group. All the rats were then killed, and the major organs (liver, spleen, heart, lungs, thymus, kidneys, adrenals, thyroid, testes, prostate, seminal vesicles, ovaries and uterus) were excised, blotted dry, and weighed. The organs from two males and two females from each group dosed at 50 mg. Fe/Kg. were examined histologically.

The group mean increases in bodyweight after 12 weeks are given in table 1. Analysis of the combined data showed that at the higher dose level all four compounds significantly depressed growth rate in the male rats, but not in the females. At the lower dose level the depressions produced in the males by the fumarate and the gluconate were not significant ($P = 0.05$). None of the organ weights (expressed in mg./100 Gm. bodyweight) in the dosed groups differed significantly ($P = 0.05$) from those of the controls. Likewise no abnormalities were found in the red or total white cell counts or hemoglobin concentrations.

Apart from a slight and variable increase in iron deposition in the tissue phagocytes (e.g., Kupffer cells, pulmonary macrophages and adrenal cortical littoral cells), histologic examination of the organs listed above revealed no abnormalities that could be attributed to the drugs.

Emetic Activity in Cats

The emetic activities of the four ferrous compounds were compared in cats, using the method of Hoppe, Marcelli and Tainter (1955).

Five adult cats, weighing between 1.8 and 3.5 Kg., were deprived of food, but not water, for 18 hours, when they were dosed orally with gelatin capsules containing the iron compounds. No animal was dosed more than twice a week. A random order of dosing was used, so that no animal received two consecutive doses of the same iron salt. After dosing, the cats were observed for emesis, which was evaluated on an "all or none" basis.

The results, together with the AED_{50} values (approximate dose producing emesis in 50 per cent of the cats), are given in table 2. It will be seen that the relative emetic activities were fumarate 1, gluconate and succinate 3, and sulphate 4.

Irritant Effects on Gastric Mucosa in Rabbits

Sixty-four adult rabbits having free access to food and water were dosed orally with tablets of ferrous fumarate, ferrous sulphate compound, ferrous succinate or ferrous gluconate. The dose was 450 mg. Fe/Kg., the rabbits receiving seven tablets of fumarate or sulphate compound, or twelve tablets of succinate or gluconate per Kg. The fumarate and sulphate tablets contained 65 mg. Fe, the succinate and gluconate tablets 36 mg. The gluconate tablets were broken to facilitate administration; the other tablets were administered whole.

The tablets were administered at 10 p.m. and the mortalities were recorded at 10 a.m. next morning. The surviving rabbits were then killed, and the stomachs and livers from all the animals were examined macroscopically and those from three of each group histologically. The macroscopic changes in the gastric mucosa were classified according to the scheme in table 3, which includes the mortality figures.

The histologic findings were as follows.

Sulphate.—Acute gastritis was present in all the rabbits, with much iron impregnation of the mucosa. Iron was present in the mucosal, submucosal and subserous vessels; it was either dissolved in the plasma or precipitated on the endothelium. Two stomachs showed early necrosis of the superficial part of the mucosa. All the livers manifested a "chemical hepatitis," the essential features of which were iron impregnation of parenchymal cells, their invasion and replacement by polymorphs, and an increase in intravascular polymorphs.

Gluconate.—The stomach of one revealed iron incrustation of the mucosa, while that of another showed superficial mucosal necrosis and iron in the vessels. In both, the pylorus showed early inflammation. Iron impregnation was apparent in the lamina propria of one, and there was iron in the vessels of the other. The livers showed a hepatitis similar in appearance to that observed in the group dosed with ferrous sulphate.

Succinate.—one pylorus showed some iron impregnation of the lamina propria and an incipient erosion. One liver showed a slight excess of polymorphs in the sinusoids.

Fumarate.—the only abnormality was observed in one of the livers, in which there were small, scattered focal necroses. The stomachs showed no inflammation or erosion, and no iron impregnation of the mucosa.

In a second experiment, four groups of three adult rabbits were dosed orally with tablets of the four iron compounds. As before, the dose employed was 450 mg. Fe/Kg. On this occasion none of the animals died within 12 hours of being dosed. They were all

Table 3.—*Effects of 4 Different Iron Tablets on Rabbit Gastric Mucosa* (12 hours after giving a single dose equivalent to 450 mg. Fe/Kg.)

Effects observed	Ferrous fumarate	Ferrous sulphate compound	Ferrous gluconate	Ferrous succinate
None	13/21	1/25	2/9	3/9
Slight inflammation	6/21	1/25	1/9	5/9
Severe and extensive inflammation	2/21	11/25	3/9	1/9
Death within 12 hours	0/21	12/25	3/9	0/9

killed at this stage, and their stomachs were removed, washed with saline, and photographed (fig. 1).

Hematinic Activity in Iron-Deficient Rats

Preliminary experiments in iron-deficient rats showed that a daily oral dose of 0.1 mg. Fe per rat induced adequate but submaximal responses in growth rate and hemoglobin production; this finding applied to all four iron compounds. Hence this dose was employed in the experiment below.

Forty-three iron-deficient albino weanling rats of the WAG strain were divided into five groups of eight and a control group of three. All the rats were housed in aluminum containers and maintained entirely on cows' milk fortified with salts (NaCl, 1 Gm.; $CuSO_4 5H_2O$, 0.6 mg.; $MnSO_4 5H_2O$, 0.6 mg. per liter). The four iron compounds were administered orally using all-glass syringes. The rats in the fifth group were given daily intramuscular injections of an iron-dextran preparation (Imferon, Bengers) diluted to contain the required dose in 0.1 ml. All five iron compounds were administered once daily on 42 consecutive days at a dose level of 0.1 mg. Fe in 0.1 ml./rat.

Individual bodyweights and hemoglobin concentrations were recorded before beginning treatment and at weekly intervals thereafter.

All three control rats died within 10 days of starting the experiment, and two of the gluconate-treated group and four of the succinate group died during the experiment.

The group mean bodyweights and hemoglobin concentrations of the survivors are given in table 4, and figure 2 shows the group mean values for total mg. hemoglobin per rat weekly throughout the dosing period. These latter values, which reflect increases in both blood volume and hemoglobin concentration, were calculated on the assumption that blood volume was 6.7 per cent of the bodyweight, i.e.,

$$\text{total mg. Hgb./rat} = \text{bodyw't in Gm.} \times \frac{6.7}{100} \times \frac{\text{Hgb. conc'n. in Gm./100 ml.} \times 1000}{100}.$$

Human Studies

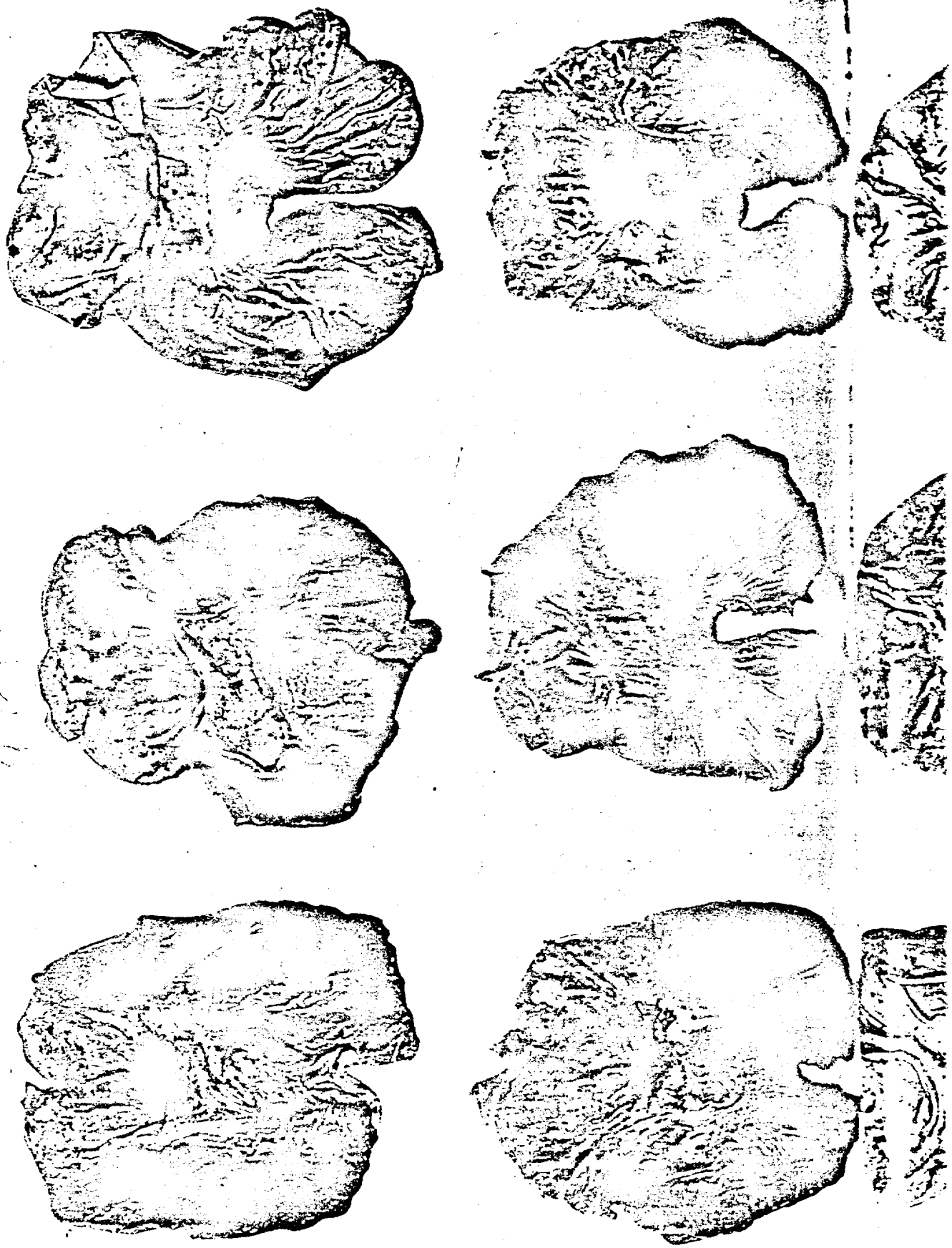
Twenty-two patients with hypochromic anemia were treated with one tablet of ferrous fumarate (Fersamal, Glaxo) three times a day (200 mg. Fe

Table 4.—*Effects of 5 Iron Compounds on Body Weights and Hemoglobin Levels of Iron-Deficient Rats*

(All dosed rats received 0.1 mg. Fe/rat/day, Imferon being administered intramuscularly, the remaining compounds orally)

Dosing period in weeks	Group mean values ± S.E											
	Controls (3)*		Fumarate (8)		Sulphate (8)		Gluconate (6)		Succinate (4)		"Imferon" (8)	
	BW.†	Hgb.‡	BW.	Hgb.	BW.	Hgb.	BW.	Hgb.	BW.	Hgb.	BW.	Hgb.
0	34 ± 4.0	5.8 ± 0.2	30 ± 2.0	5.1 ± 0.4	31 ± 2.3	5.0 ± 0.5	30 ± 2.3	6.3 ± 0.3	28 ± 2.8	6.7 ± 0.3	33 ± 1.9	5.6 ± 0.5
1	35 ± 3.6	4.9 ± 0.5	40 ± 2.3	8.4 ± 0.7	41 ± 2.1	8.2 ± 0.7	33 ± 2.7	8.5 ± 0.4	32 ± 3.3	8.2 ± 0.7	38 ± 2.1	6.8 ± 0.6
2	All dead		48 ± 3.9	8.9 ± 0.6	51 ± 2.6	8.7 ± 0.5	38 ± 2.7	8.3 ± 0.6	31 ± 1.7	8.2 ± 1.9	47 ± 2.8	7.1 ± 0.4
3			59 ± 4.7	10.6 ± 0.4	60 ± 3.5	10.4 ± 0.5	36 ± 2.9	9.4 ± 1.1	36 ± 2.6	7.4 ± 1.1	53 ± 2.8	8.8 ± 0.5
4			71 ± 7.2	11.8 ± 0.5	70 ± 4.3	11.9 ± 0.5	43 ± 4.6	10.5 ± 0.7	47 ± 5.8	8.6 ± 0.8	63 ± 2.6	11.7 ± 0.6
5			80 ± 8.8	12.5 ± 0.6	83 ± 3.9	12.2 ± 0.6	54 ± 5.3	10.7 ± 1.0	67 ± 7.1	9.8 ± 1.3	76 ± 3.6	13.1 ± 0.6
6			91 ± 9.3	12.4 ± 0.2	95 ± 4.3	12.8 ± 0.4	61 ± 5.4	12.8 ± 1.1	80 ± 9.7	10.9 ± 0.9	88 ± 5.1	14.2 ± 0.6

*( ) = number of rats in group.
†B.W. = body weight in Gm.
‡Hgb. = g. hemoglobin %.



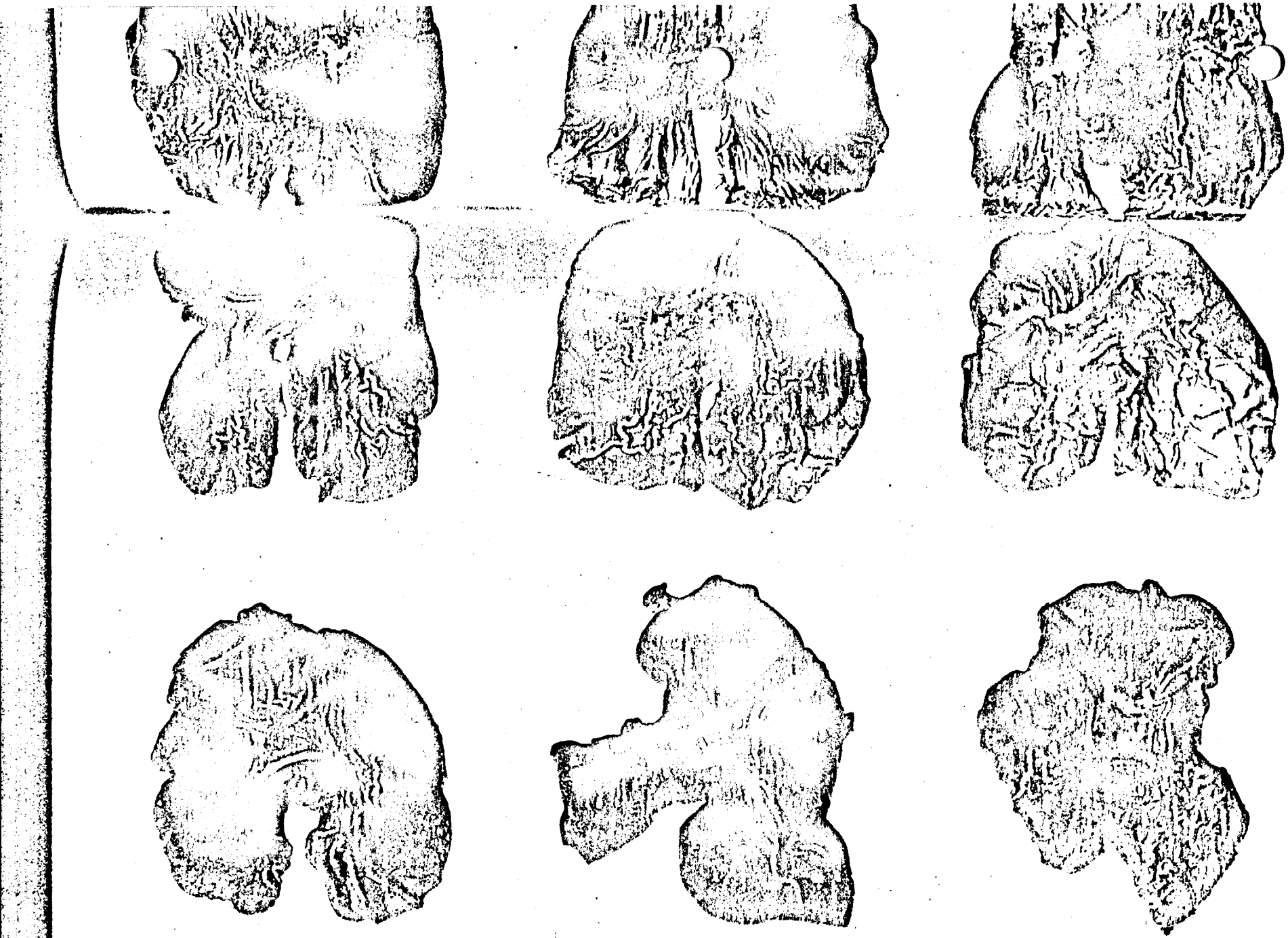


Fig. 1.—The effects of four different iron tablets on rabbit gastric mucosa. (Single oral dose of 450 mg. Fe/Kg. body weight administered 12 hours before.) *Page* 544—*Top*, ferrous gluconate compound; *bottom*, ferrous sulphate. *Page* 545—*Top*, ferrous fumarate; *bottom*, ferrous succinate.

Fig. 1.—*Continued.* Controls, not dosed.

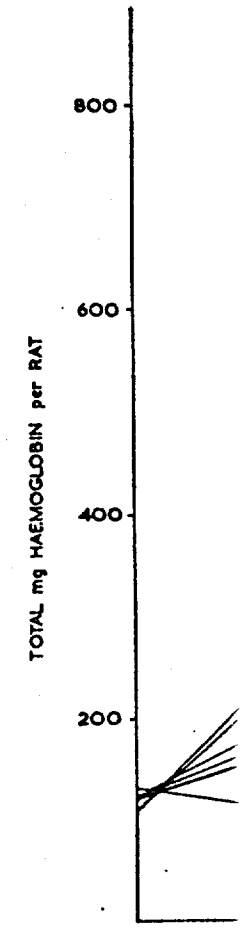


Fig. 2.—The hem
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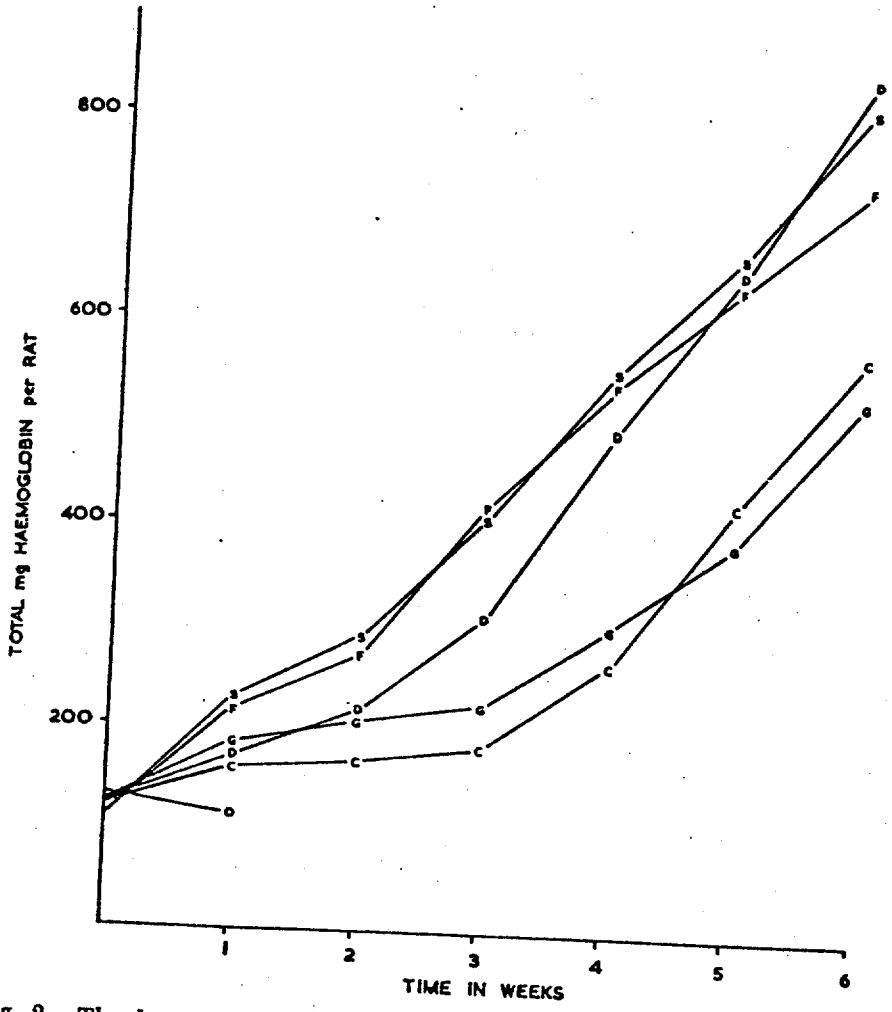

Fig. 2.—The hematinic effects of five iron compounds on iron-deficient rats. Dose, 0.1 mg. Fe/rat/day. O, controls, no iron; D, iron dextran, intramuscular; C, ferrous succinate, oral; G, ferrous gluconate, oral; S, ferrous sulphate, oral; F, ferrous fumarate, oral.

per day). The product was acceptable to all the patients, and, apart from one who had undergone gastrectomy, all showed hematologic improvement. The patient who proved refractory had not previously responded to ferrous sulphate or ferrous gluconate.

The cases were divided into two categories with initial hemoglobin levels above or below 50 per cent (7.4 Gm. hemoglobin per 100 ml.). The results are given in figures 3 and 4, respectively.

As would be expected, the most rapid gains in hemoglobin occurred in the more severely anemic subjects. After treatment for 30 days, there were average gains in hemoglobin (Gm./100 ml./day) of 0.105 in the first group (fig. 3) and 0.170 in the second (fig. 4).

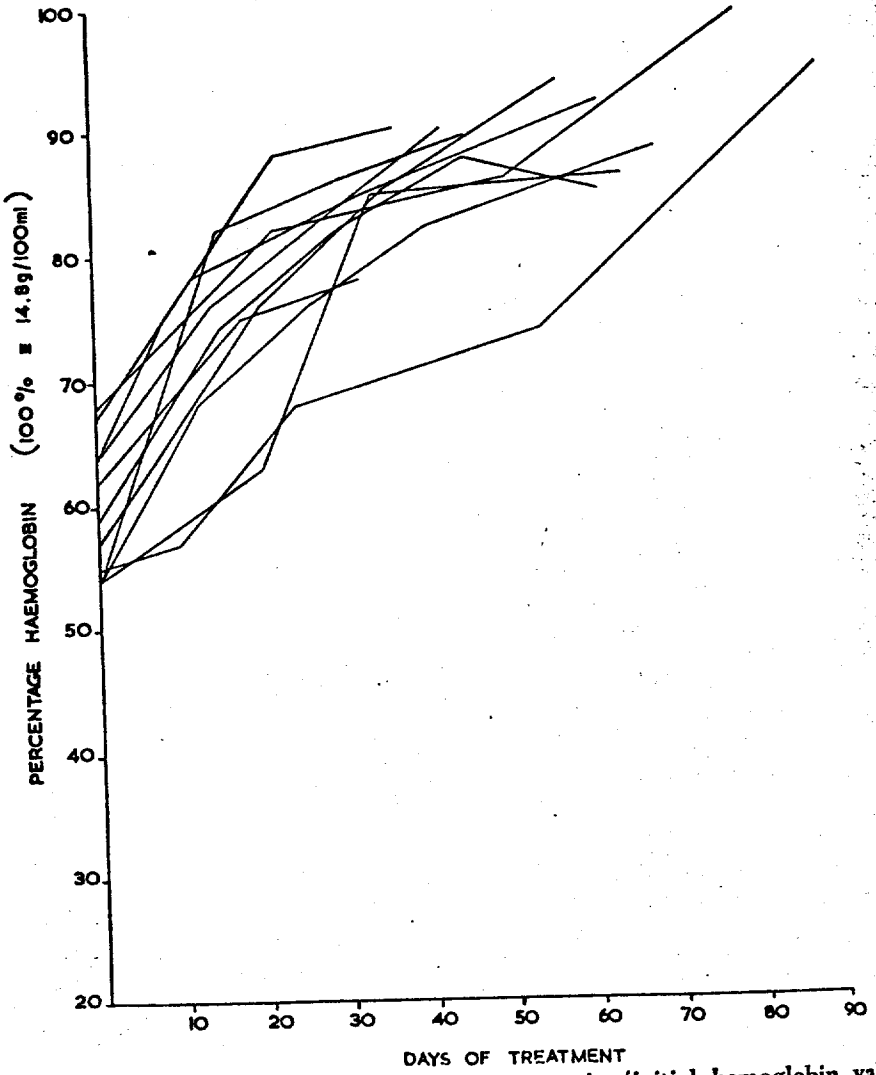

Fig. 3.—Eleven patients with hypochromic anemia (initial hemoglobin values above 50 per cent) dosed with ferrous fumarate (200 mg. three times daily).

Summary

Ferrous fumarate, an oral hematinic, has been compared with the sulphate, succinate and gluconate for various aspects of toxicity.

In mice, the relative acute oral toxicities were fumarate 1, succinate 1.1, gluconate 2.0 and sulphate 2.7.

In cats, the relative emetic activities were fumarate 1, succinate and gluconate 3, and sulphate 4.

Examination of the stomachs and livers of rabbits given massive doses of the four iron tablets showed that the sulphate and gluconate were much more toxic and irritant than the succinate or fumarate.

Rats dosed for 12 weeks with ferrous fumarate (50 mg. Fe/Kg./day) grew

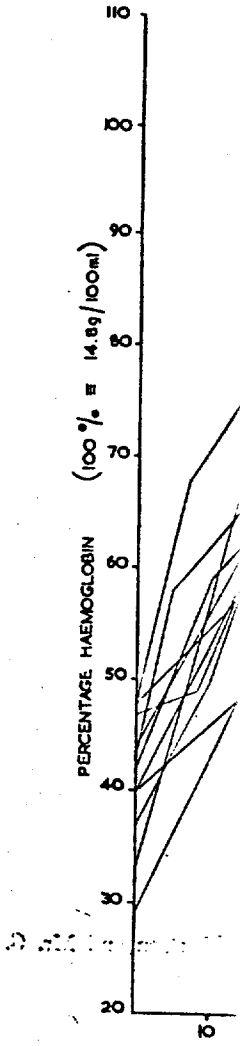

Fig. 4.—Eleven
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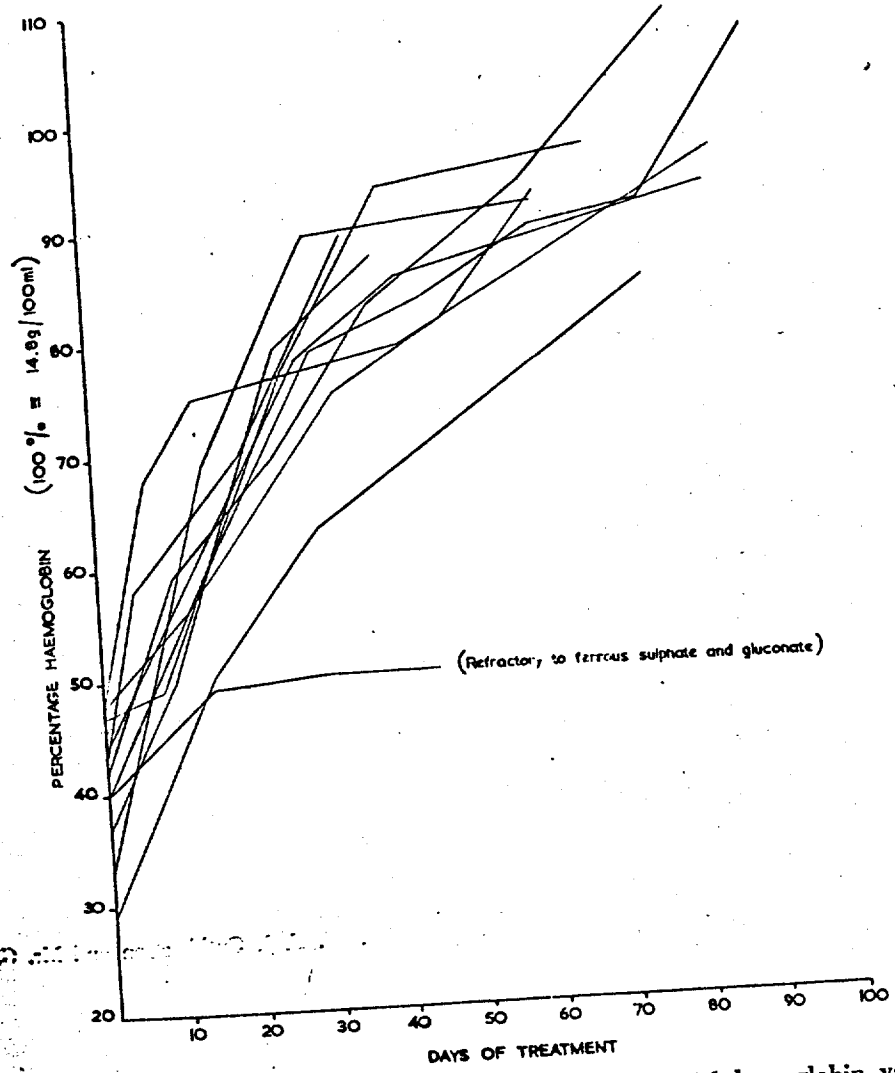

Fig. 4.—Eleven patients with hypochromic anemia (initial hemoglobin values below 50 per cent) dosed with ferrous fumarate (200 mg. three times daily).

normally, and histologic examination of the major organs revealed no abnormalities that could be attributed to the drug.

Hematinic studies on iron-deficient rats, receiving the four iron compounds orally or iron-dextran intramuscularly, indicated that ferrous fumarate was as effective as the other compounds.

Twenty-two hypochromic anemic patients were dosed with Fersamal (three tablets per day), and all except one showed hematologic improvement. The patient who proved refractory had previously undergone gastrectomy and had not improved on either ferrous sulphate or gluconate.

The tablets were acceptable to all the patients.

Summiario in Interlingua

Le hematinico oral, fumarato ferrose, esseva comparate con respecto a varie aspectos de toxicitate con le correspondente sulphato, succinato, e gluconato.

In muses, le relative toxicitates oral esseva 1 pro fumarato, 1,1 pro succinato, 2,0 pro gluconato, e 2,7 pro sulphato.

In cattos, le relative activitates emetic esseva 1 pro fumarato, 3 pro succinato e gluconato, e 4 pro sulphato.

Le examine del stomachos e del hepates de conilios tractate con doses massive del quatro compositos de ferro in le forma de comprimitos oral monstrava que le sulphato e le gluconato esseva multo plus toxic e irritante que le succinato o le fumarato.

Rattos tractate durante 12 septimanas con fumarato ferrose in un dosage de 50 mg de ferro per kg de peso corporee per die cresceva normalmente, e le examine histologic del organos major revelava nulle anormalitates que poteva esser attribuite al effecto del droga.

Studios hematinic in rattos can deficientia de ferro que esseva tractate con le quatro compositos de ferro per via oral o con ferro e dextrano per via intramuscular indicava que fumarato ferrose esseva tanto efficace como le altere compositos.

Vinti-duo patientes con anemia hypochromic esseva tractate con Fersamal (tres comprimitos per die), e omnes—con un exception—monstrava un melioration hematologic. Le patiente qui se provava refractori habeva previemente essite subjicite a gastrectomia e habeva monstrate nulle melioration sub tractamentos con sulphato o gluconato ferrose.

Le comprimitos esseva acceptabile pro omne le patientes.

ACKNOWLEDGMENTS

We wish to thank Mrs. Carole Bedford, Miss Patricia Sutherland, Mr. J. Dunnington and Mr. C. Robinson for technical assistance, and Dr. W. F. J. Cuthbertson and Mr. G. Flynn, who supplied the iron-deficient rats. The photography was carried out by Mr. D. F. Boxall.

The clinical studies included in this paper were carried out by J. N. Marshall Chalmers, M.D., F.R.C.P., Queen Elizabeth Hospital, Birmingham.

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IRON ABSORPTION BY WOMEN: COMPARISON OF THREE FERROUS SALTS

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LINCOLN, NEB.

A NEED exists for specific information on the absorption, by human subjects, of iron from different iron salts which are used medicinally or in the enrichment of certain foods. Results of studies on rats and dogs are not completely applicable to man.

In man the absorption of iron from the gastrointestinal tract is normally controlled by his need for it. A person who is deficient in iron or who has an increased need, as in growth, pregnancy, and lactation, absorbs a greater proportion of the iron from the daily intake than does a normal person.

The study being reported was planned to compare the iron absorption and hemoglobin response of women given iron in the form of ferrous gluconate, ferrous lactate, or ferrous sulfate. Healthy nonanemic women were chosen for subjects in order to control in so far as possible the effect of iron need on iron absorption and thus reduce variability among the subjects. Eighty-three college students and staff members ranging in age from 18 to 42 years and living on self-selected diets served as subjects.

METHODS

For a period of four weeks, ferrous gluconate was given daily to twenty-seven of the women, ferrous lactate to twenty-nine, and ferrous sulfate to twenty-seven. The daily iron medication was: ferrous gluconate, 99 mg.; ferrous lactate 102 mg.; and ferrous sulfate, 101 mg. Capsules containing the iron salt were made especially for the study, and the maximum variation in the iron content of different capsules containing the same salt was 1 per cent, or 1 mg. The subjects took the capsules with the evening meal, and did not eat liver nor spinach during the month of medication.

Complete fecal collections were made during the third and fourth weeks of medication, that is, from the fourteenth through the twenty-eighth day.

The authors have had extensive experience with the analysis of biological materials for iron. Extreme precautions are routinely taken to avoid contamination and to insure recoveries between 98 and 102 per cent of quantitatively added iron (Leverton[1]). The fecal excretions of each subject for the two-week period were combined and made into a slurry with 10 per cent HCl, then an aliquot was ashed at 500° C., just below ''red'' heat. The white ash was dissolved in HCl, and the iron in it was determined by the method of Pohle and associates.[2] The optical density of the colored complex formed by 1,10-phenanthroline and the reduced iron was measured in a Beckman spectrophotometer for which the extinction coefficient of iron had been determined.

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From the Human Nutrition Research Laboratory, Agricultural Experiment Station, Lincoln, Neb. The work was supported in part by a grant from the Smith Dorsey Company, Lincoln.

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for four weeks. Iron absorption, or the difference between intake and fecal excretion, was measured during the third and fourth weeks of medication, and hemoglobin values were determined weekly during medication and for four weeks thereafter. The mean daily absorption of the subjects who were given ferrous gluconate was 9 mg., ferrous lactate 13 mg., and ferrous sulfate 11 mg. The difference between these means was not statistically significant. However, there was some indication that the women absorbed iron from ferrous lactate somewhat more consistently than from ferrous gluconate or ferrous sulfate. The hemoglobin of these normal women did not increase with iron medication.

TABLE I. MEAN DAILY INTAKE* AND ABSORPTION OF IRON DURING THIRD AND FOURTH WEEKS OF IRON MEDICATION

	FERROUS GLUCONATE		FERROUS LACTATE		FERROUS SULFATE	
	MEAN	S.D.†	MEAN	S.D.	MEAN	S.D.
All subjects	27		29		27	
Intake Fe (mg.)	109		112		111	
Absorption (mg.)	9	18.9	13	13.2	11	10.0
Group 1						
Subjects storing iron	14		21		17	
Per cent of subjects	52		72		63	
Absorption (mg.)	24	13.6	19	9.9	16	8.3
Group 2						
Subjects losing iron	8		3		1	
Per cent of subjects	29		10		4	
Absorption (mg.)	-11	6.4	-8	2.7	-6	0
Group 3						
Subjects in equilibrium	5		5		9	
Per cent of subjects	19		17		33	
Absorption (mg.)	-1	3.2	0	2.7	2	1.9

*The self-selected diets supplied approximately 10 mg. of iron daily.
†Standard deviation.

TABLE II. MEAN HEMOGLOBIN VALUES BEFORE, DURING, AND AFTER FOUR WEEKS OF IRON MEDICATION

TIME OF TEST	FERROUS GLUCONATE		FERROUS LACTATE		FERROUS SULFATE	
	MEAN	S.D.*	MEAN	S.D.	MEAN	S.D.
Number of subjects	27		29		27	
Initial† value, gm. per cent	13.4	0.65	13.2	0.73	13.1	0.69
Week of medication						
1	13.3	0.79	13.2	0.77	13.1	0.67
2	13.3	0.90	13.1	0.73	13.1	0.73
3	13.3	0.77	13.3	0.84	13.4	0.65
4	13.2	0.87	13.3	0.88	13.3	0.84
Week after medication						
1	13.4	0.69	13.2	0.75	13.3	0.83
2	13.5	0.84	13.5	0.91	13.3	0.66
3	13.4	0.93	13.4	0.91	13.3	0.88
4	13.4	0.82	13.3	0.76	13.4	0.69

*Standard deviation.
†Average of two values on consecutive days.

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Hemoglobin determinations were made in duplicate for two consecutive days before medication began and weekly thereafter until four weeks after medication had been discontinued. To avoid diurnal variation in hemoglobin values the venous blood samples for each subject were taken at the same time of day for each of the tests. Hemoglobin was converted to oxyhemoglobin in 0.1 per cent sodium carbonate, and the light transmittance of the solution was measured in the Sheard-Sanford photelometer.[3]

RESULTS

The mean daily intakes and absorptions of iron during the third and fourth weeks of iron medication are shown in Table I. The mean daily iron absorption by subjects given ferrous gluconate was 9 mg. (S.D. = 18.9), ferrous lactate 13 mg. (S.D. = 13.2), ferrous sulfate 11 mg. (S.D. = 10.0). The absorption was small because the need as evidenced by the normal hemoglobin values was small. Analysis of variance did not show a significant difference among these means.

For further analysis of the data the subjects were sorted into three groups on the basis of individual performance and the mean absorption of each group is also presented in Table I. Group 1 includes those who stored more than 5 mg. of iron daily; Group 2, those who excreted more than 5 mg. in excess of the amount they were ingesting; and Group 3, those who neither stored nor lost more than 5 mg. of iron daily and therefore were considered to be in equilibrium.

This rather generous interpretation of equilibrium allows for some of the vagaries of the digestive tract in the absorption and excretion of iron. Even among healthy individuals there are differences in gastric acidity, intestinal motility, and iron requirement which cause variation in iron absorption.

Sixty-three per cent of all the subjects were storing iron, 23 per cent were in equilibrium, and 14 per cent were losing iron from the body. The loss of iron by 14 per cent or twelve of the subjects when on daily intakes as high as 110 mg. of iron was unexpected, and is not easily explained. Diarrhea did not occur among these subjects.

Although only small differences in the absorption of iron from the three ferrous salts were observed, more subjects absorbed iron from ferrous lactate than from the gluconate or sulfate. Seventy-two per cent of the women given ferrous lactate stored iron and their mean daily absorption was 19 mg. (S.D. = 9.9). In contrast to this, only 52 per cent of the women given ferrous gluconate stored iron, however, their mean daily absorption was 24 mg. (S.D. = 13.6); and 63 per cent of the women given ferrous sulfate stored iron with a mean daily absorption of 16 mg. (S.D. = 8.3).

The means and standard deviations of the hemoglobin values for all subjects given each iron salt are shown in Table II. Hemoglobin values did not change significantly during the four weeks of the medication nor during the four weeks after medication had ceased. Nor was there any difference in the hemoglobin response of the subjects who were storing iron as compared with those in equilibrium or with those who were losing iron.

SUMMARY

Twenty-seven normal women were given ferrous gluconate, 29 ferrous lactate, and 27 ferrous sulfate at a level of approximately 100 mg. of iron daily

We intend to incorporate this method into the practical side of a growth study, as it will not only greatly increase our accuracy but provide us with permanent records of the tissue growth.

We are grateful to Dr. T. H. Hills and Dr. E. A. Miskin for many helpful suggestions.

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THE THERAPEUTIC RESPONSE OF SECONDARY ANAEMIAS TO ORGANIC AND INORGANIC IRON SALTS

BY

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In 1926 Starkenstein discovered that only ferrous iron is utilizable by the haemopoietic system. Since then inorganic ferrous salts have provided the standard form of ferrotherapy. In particular, ferrous sulphate has been widely used in various official and proprietary preparations. The *National Formulary 1952* informs us that there is need for only two oral preparations of iron in the treatment of ferro-sensitive anaemias. These are those containing either ferrous sulphate or iron and ammonium citrate.

Benstead and Theobald (1952), in their work on the incidence of anaemia in pregnancy, showed in their series of cases that in one group of patients 33%, and in another group 40.2%, found it difficult or impossible to tolerate ferrous sulphate tablets. They concluded that 30–40% of antenatal patients do not, in fact, consume these tablets when prescribed as routine in antenatal departments. It therefore seems that, although ferrous sulphate tablets are widely ordered, their therapeutic value, owing to gastric intolerance, leaves much to be desired.

Following their publication I wrote (Haler, 1952) that I was investigating the therapeutic effects of the organic ferrous gluconate in ferro-sensitive anaemias. The results are herewith appended.

Ferrous gluconate is the normal ferrous salt of D-gluconic acid; it is a dihydrate crystal and contains 11.5% of ferrous iron.

Staub (1949), Jasinki (1949), and others have shown that this salt is the most easily absorbed of all ferrous salts. It does not appear to produce gastric upsets. The following figures result from the decision to compare the therapeutic response of ferro-sensitive anaemias when treated with the standard inorganic iron preparations with those obtained from the use of ferrous gluconate.

The Investigation

The cases investigated are classified as follows:

Anaemia following	post-partum haemorrhage ..	25 cases
"	normal delivery	13 "
"	illness	6 "

TABLE I

Code	No. of Cases	Key	Daily Dosage of Available Iron
		Group A. Inorganic Iron Preparations	
A	5	Proprietary ferrous sulphate and folic acid in capsules	184 mg.
B	1	Proprietary ferrous sulphate capsules	184 "
C	11	" " "	180 "
D	1	Tab. ferrous sulphate Co. *N.F.* " ..	180 "
F	1	Ferri dia'ysata	216 "
H	1	Proprietary saccharated iron for intravenous use	100 "
J	1	Proprietary iron and folic acid tablets	184 "
		Group B. Organic Iron Preparations	
I	23	Ferrous gluconate	105 "

The cases were selected at random, the average Hb value (King and Gilchrist, 1947; King *et al.*, 1947, 1948a, 1948b, 1948c, 1951; Macfarlane *et al.*, 1948; Donaldson *et al.*, 1951) over the series being 65%; organic and inorganic iron preparations were given to alternate cases. The organic preparation of iron was available as a liquid proprietary preparation which in addition contained aneurin hydrochloride, nicotinamide, and riboflavin in blackcurrant juice syrup, which also provided 5 mg. of natural vitamin C per drachm.

In Group A cases (Table I) supplementary vitamins were given to those patients in whom avitaminosis was suspected.

TABLE II

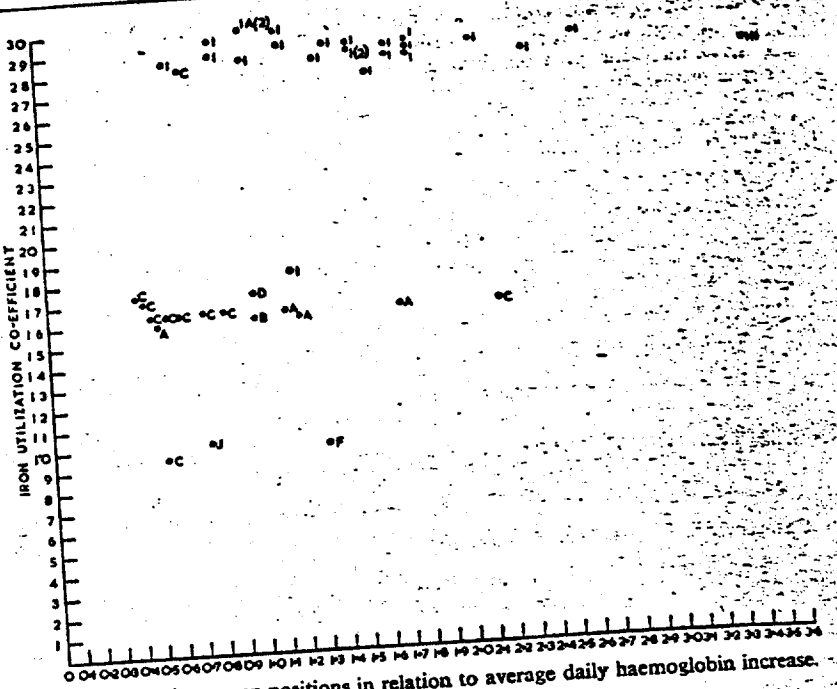
Case No.	Group	Hb Initial	Hb Final	Hb Increase	Hb Mean Daily Increase%	Total Fe++ Administered	% Fe++ Utilized	Coefficient
				Group A. Inorganic Iron Preparations				
1	A	62 (9·25)	90 (13·5)	28 (4·25)	1·16	4,416	19·02	16·4
2	A	70 (10·45)	92 (13·80)	22 (3·35)	0·54	7,554	8·7	16·1
3	A	80 (12·0)	86 (12·9)	6 (0·90)	1·2	920	19·6	16·3
4	A	68 (10·15)	80 (12·0)	12 (1·85)	1·5	1,288	28·0	16·45
5	A	32 (4·75)	41 (6·1)	9 (1·35)	1·0	900	30·0	30·0
6	C	52 (7·7)	74 (11·05)	22 (3·35)	2·2	1,800	36·0	16·4
7	C	60 (9·0)	76 (11·4)	16 (2·4)	0·67	2,880	18·8	28·0
8	C	54 (8·05)	68 (10·15)	14 (2·1)	0·67	3.780	11·1	16·6
9	C	70 (10 5)	90 (13·5)	20 (3·0)	0·57	6.300	9·5	16·7
10	C	68 (10·15)	90 (13·5)	22 (3·35)	0·43	8,820	7·5	17·4
11	C	48 (7·2)	72 (10·7)	24 (3·5)	0·84	5.580	13·9	16·6
12	C	68 (10·15)	82 (12·3)	14 (2·15)	0·5	5,040	8·3	16·6
13	C	34 (5·00)	76 (11·4)	42 (6·4)	0·875	8,640	14·5	16·7
14	C	58 (8·65)	84 (12·6)	26 (3·95)	0·74	6,300	12·4	16·75
15	C	60 (9·0)	78 (11·7)	18 (2·7)	0·47	13,680	4·0	8·5
16	C	50 (7·5)	58 (8·65)	8 (1·15)	0·53	5,400	4·5	8·5
17	B	76 (11·4)	80 (12·0)	4 (0·60)	1·0	736	16·3	16·3
18	D	48 (7·2)	70 (10·5)	22 (3·30)	1·0	7,560	8·8	8·8
19	F	45 (6·7)	75 (11·2)	30 (4·5)	1·4	4,536	13·9	10·0
20	H	41 (6·1)	58 (8·65)	17 (2·55)	3·4	525	97·0	28·6
21	J	55 (8·2)	82 (12·3)	27 (4·10)	0·77	6,440	7·9	10·2
				Group B. Organic Iron Preparation				
23	I	62 (9·2)	92 (13·8)	30 (4·6)	1·67	1,890	47·6	29·5
24	I	58 (8·65)	92 (13·8)	34 (5·15)	1·88	1,890	52·9	28·0
25	I	76 (11·4)	90 (13·5)	14 (2·1)	1·2	1.890	22·2	18·5
26	I	72 (10·7)	94 (14·1)	22 (3·4)	1·8	1,890	50·8	28·2
27	I	70 (10·5)	98 (14·85)	28 (4·35)	1·6	1,890	44·4	27·8
28	I	80 (12·0)	98 (14·85)	18 (2·85)	1·0	1,890	28·6	28·6
29	I	47 (7·0)	84 (12·6)	37 (5·6)	1·8	2,205	50·2	27·9
30	I	80 (12·0)	98 (14·85)	18 (2·85)	1·5	1,260	42·9	28·6
31	I	54 (8·0)	92 (13·8)	38 (5·8)	2·1	1,890	60·3	28·8
32	I	72 (10·7)	90 (13·5)	18 (2·8)	2·6	735	75·2	28·9
33	I	72 (10·7)	88 (13·2)	16 (2·5)	1·33	1,260	38·1	28·7
34	I	77 (11·5)	95 (14·2)	18 (2·7)	1·5	1,260	42·9	28·6
35	I	76 (11·4)	96 (14·4)	20 (3·0)	1·67	1,890	47·6	28·5
36	I	78 (11·7)	96 (14·4)	18 (2·7)	2·33	1,260	66·6	28·6
37	I	68 (10·15)	90 (13·50)	22 (3·35)	1·2	1,890	34·9	29·0
38	I	78 (11·7)	93 (14·0)	15 (2·3)	1·5	1,050	42·8	28·8
39	I	90 (13·5)	96 (14·4)	6 (0·9)	0·85	735	25·0	29·4
40	I	60 (9·0)	82 (12·3)	22 (3·3)	1·8	1,260	52·4	29·0
41	I	74 (11·05)	96 (14·4)	22 (3·35)	1·0	2,205	30·0	30·0
42	I	70 (10·5)	94 (14·1)	24 (3·6)	0·85	2,940	24·5	28·8
43	I	72 (10·7)	98 (14·85)	26 (4·15)	0·62	4,410	17·7	28·6
44	I	65 (9·7)	100 (15·0)	35 (5·3)	1·2	2,940	35·7	29·8
45	I	68 (10·15)	78 (11·7)	10 (1·55)	1·4	735	40·8	29·2

		Available Fe++/day
A	= "Plastules" with folic acid	184 mg.
B	= "Plastules" plain	184 "
C	= "Ferso'ate"	180 "
D	= Tab ferri sulph. co.	180 "
F	= Ferri dia'ysat.	216 "
G	= Ferri ammon. cit.	189 "
H	= "Ferrivenin"	100 "
I	= "Cerevon"	105 "
J	= "Folvite" with iron ("Folvron") ..	184 "

Assessment of the therapeutic response was based on the following factors: (1) total increase in grammes of Hb per 100 ml.; (2) average daily Hb increase per 100 ml. (taking 15 g. Hb as 100%); (3) the iron utilization coefficient, calculated on the basis that 30 mg. of iron is required to raise the Hb by 1%; and (4) the average number of treatment-days required to raise the Hb value to within normal limits. Routine haematological investigation was carried out before and during treatment, and the results were carefully recorded.

Study of the Therapeutic Response

Group A (Table II) shows the results of treatment in those cases treated with inorganic iron. The average total dosage of available iron administered was 4,900 mg. The average Hb increase in this group was 2.9 g. per 100 ml. It can therefore be deduced that the iron utilization coefficient for this group was 18.1. This approximates to the findings of Witts (1936) for the iron utilization coefficient of ferrous sulphate as 14. The mean daily Hb increase was 1.02%, which confirms the therapeutic response to be expected. The average period of treatment over this group was 21.7 days.



Graph showing group positions in relation to average daily haemoglobin increase.

In Group B the average total dosage of available organic iron given was 1,359 mg. The average Hb increase was 3.4 g. per 100 ml., indicating an iron utilization coefficient of 28.3. This compares favourably with the finding of Staub following his study of ferrous gluconate in tablet form, which showed an iron utilization coefficient of 22±5%. The mean daily Hb increase in this group was 1.49% and the average period of treatment 17.8 days.

The analysis of the haemoglobin response in both the groups indicates that the organic iron-treated group shows a remarkably constant iron utilization coefficient of 28.3. This compares with the inorganic iron-treated group, which shows an average utilization coefficient of 18.1. This increased haemoglobin response was achieved in a shorter period of time, and with a reduced overall dosage of iron.

An Interesting Case Noted During Study

Case 20.—A full report on this case of post-natal anaemia following post-partum haemorrhage is given, as iron therapy was started with intravenous iron and completed with the ferrous gluconate preparation. The findings were as follows:

	Date	R.B.C. (mill.)	Hb Haldane (%)	C.I.	M.C.D. (μ)	Retics. %	Retics. Total
Ferrivenin, 100 mg. daily ..	20/5/52	2·0	32	0·8	6·85	0·6	12,000
	23/5/52	2·1	34	0·81	6·8	0·6	12,000
Ferrous gluconate, 105 mg., available iron daily ..	27/5/52	2·5	41	0·82	6·85	0·45	10,000
After 5 days	31/5/52	3·12	58	0·92	6·9	1·4	43,000
„ 10 „	9/6/52	4·32	74	0·84	6·7	1·9	82,000

Tolerance and Side-effects

It is usually stated that ferrous sulphate tablets are well tolerated. I first learnt to doubt this when I was treated for anaemia arising during a severe pneumonia. My gastric experiences during that treatment led me to watch for other people's intolerance to iron. It has been found that quite 25% of out-patients treated with the standard therapy voluntarily discontinue treatment because they are unwilling or unable to tolerate the gastric side-effects.

The liquid ferrous gluconate has proved so effective that patients in the ward have asked to be taken off the tablets and to be put on to the "liquid tonic."

Summary

Of all the preparations used in this trial, the liquid ferrous gluconate preparation was far and away the most popular with patients, and produced a therapeutic response out of all expectations.

A study was made of the relative therapeutic values of organic and inorganic iron salts in the treatment of ferro-sensitive anaemia. The inorganic iron preparations provided ferrous sulphate in various presentations, the daily intake of available iron being approximately 180 mg. a day.

The organic iron salt administered was a proprietary liquid ferrous gluconate preparation giving a daily intake of available iron of 105 mg. a day, with the addition of aneurin hydrochloride, nicotinamide, riboflavin, and natural vitamin C which in the dosage given provided 15 mg. of ascorbic acid a day.

An evaluation of the therapeutic response indicates that the group treated with the inorganic iron salt showed a mean daily increase of 1.02% haemoglobin and an iron utilization coefficient of 18.1.

The group treated with the organic preparation showed a mean daily haemoglobin increase of 1.49% and an iron utilization coefficient of 28.3.

The period of treatment required to raise the haemoglobin value to normal limits was 21.7 days in the inorganic iron group and 17.8 days in the organic iron group. The average total dosage of available iron administered in the inorganic group was 4,900 mg. and in the organic group 1,359 mg.

I suggest that the organic iron salt ferrous gluconate seems to produce a more satisfactory haemoglobin response within a shorter period of time than that obtained by the well-known ferrous sulphate preparation.

I wish to acknowledge my indebtedness to my personal laboratory staff for their great and invaluable assistance with the laborious assessment of this mass of figures; to my secretarial

staff for their careful typing in this detailed work; to Messrs. Calmic for their generous supply of ferrous gluconate used in this test; to all those clinicians who have assisted so kindly in placing clinical material at my disposal; and, above all, to all patients who shared in this stimulating piece of laboriously built-up research.

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Medical Memorandum

Vasa Praevia

Vasa praevia may be defined as a condition in which the foetal blood vessels, unsupported by either umbilical cord or placental tissue, traverse the foetal membranes of the lower uterine segment in front of the presenting part. As a rule it is impossible to diagnose abnormalities of the placenta until after delivery. Vasa praevia is an exception to this rule, as on rare occasions the condition has been recognized before rupture of the vessels has occurred. It is a rare condition: a survey of the literature reveals only 52 recorded cases, and for this reason the following case is of interest as an example of an uncommon cause of stillbirth.

CASE REPORT

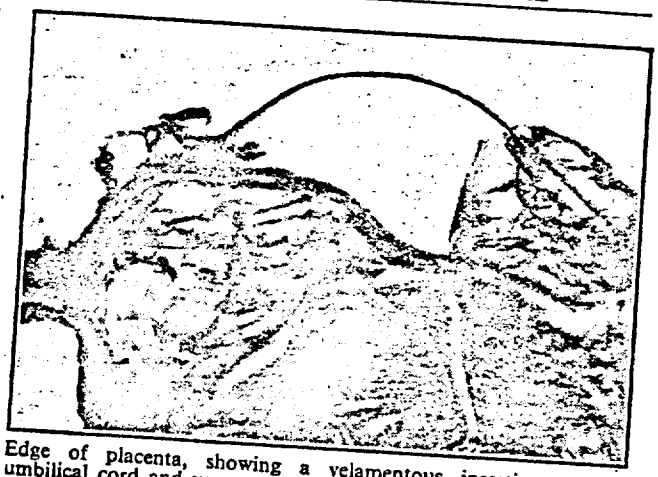
A primipara first attended the antenatal clinic on October 26, 1951. Her menses had always been regular, and her last menstrual period was on June 24. No abnormality was detected on examination, the height of the uterine fundus corresponding with an 18-weeks pregnancy. She remained well throughout pregnancy. On March 24, 1952, when she was 39 weeks pregnant, premature rupture of the membranes occurred at 5.45 a.m., and she noticed that the liquor was blood-stained. No foetal movements were felt after 6 a.m., and she was admitted to hospital at 9 a.m.

On admission she was having no pains, but was draining liquor which was mixed with a little fresh blood. The blood pressure was 140/80 and there was no albumin in the urine. On abdominal palpation the height and girth of the uterus were compatible with a full-term pregnancy. The lie of the foetus was longitudinal, and the head was deeply engaged. The uterus was not tender to palpation and foetal parts were readily felt. No foetal heart could be heard. On vaginal examination the cervix was partly effaced and one finger dilated. The membranes were ruptured and the vertex was presenting. No umbilical cord could be felt and the liquor was blood-stained. A diagnosis of revealed accidental haemorrhage was made.

Labour began at 1 p.m.; seven hours later the cervix was fully dilated. Throughout the first stage the loss of blood-stained liquor continued, and it was estimated that the total blood loss was about 120 ml. The mother had not felt any foetal movements, nor had the foetal heart been heard since admission. Forceps delivery was performed in the second stage of labour because of maternal distress. The infant was stillborn, weighing 7 lb. 2 oz. (3.2 kg.). No cord was felt on examination under anaesthesia. After delivery of the infant it was noticed that the umbilical cord was pale and that the umbilical vessels, particularly the arteries, contained very little blood. The puerperium was uneventful and the mother left hospital on the tenth day.

Placenta.—This was of normal size and the maternal surface appeared normal. There was no evidence of any retroplacental haematoma. A velamentous insertion of the cord was present, the umbilical vessels traversing the membranes for a distance of about 10 cm. before reaching the foetal surface of the placenta. One of these vessels, an artery, was divided in two by the rent in the membranes through which the baby had been delivered (see illustration).

Necropsy.—At post-mortem examination of the foetus the findings were compatible with foetal death due to anoxia consequent upon blood loss and interference with the foetal circulation. No other cause for death was found.



Edge of placenta, showing a velamentous insertion of the umbilical cord and vasa praevia. A small metal probe has been inserted into the lumen of the divided umbilical artery. (Owing to preservation of the specimen in formalin before photography, the membranes have shrunk.)

COMMENT

Velamentous insertion of the cord is almost a prerequisite for the occurrence of vasa praevia. Only very rarely may it be attributed to vessels running between the lobes of a multipartite placenta; to the vessels of a succenturiate lobe crossing the lower segment; or to a marginal insertion of the cord, in which condition aberrant vessels sometimes traverse the membranes before finally penetrating placental tissue. Rucker and Tureman (1945) state that the incidence of velamentous cord insertion has been given by various authors as between 0.4 and 0.9%. De Lee (1913) declares that there is a much higher incidence in multiple pregnancy. It is often associated with other placental abnormalities—namely, multilobed placentae, placenta succenturiata, and, in particular, placenta praevia. Although in most cases when bleeding from velamentous vessels occurs the vessels are praevia, five cases have been recorded in which vessels in the fundus have been ruptured.

The risk to the foetus is considerable, and foetal death *in utero* may occur from either asphyxia or exsanguination. Asphyxia is due to compression of the aberrant vessels in the lower segment by the presenting part. It is probably the greater of the two dangers to the foetus. Exsanguination may occur if the vessels are torn across.

Should a vasa praevia rupture, the child, even if born alive, may be severely anaemic and neonatal death may occur. The foetal loss in the 53 cases so far recorded has been 55%. This is very similar to the figure of 58% quoted by Graff (1921) in reviewing all cases up to that time. But in the 41 cases (67%) in which rupture of the vessels was known to have occurred the foetal loss was 73%. It must, however, be emphasized that vasa praevia is essentially a foetal complication. The only added hazards to the mother are those attendant upon interference by the accoucheur, such interference being undertaken solely on behalf of the foetus. Caesarean section is indicated in those cases discovered early in labour with the membranes intact, but the operation should only be considered before the vessels rupture, for once haemorrhage has occurred the prognosis for the infant is too bad to justify the increased risk to the mother. McNair (1921) and Vogt (1943) obtained living infants by abdominal delivery.

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JAMA 186, 1963

Desferrioxamine in the Treatment of Acute Toxic Reaction to Ferrous Gluconate

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Desferrioxamine, a new, specific iron chelating agent, was used in the successful treatment of a child with acute iron intoxication due to ferrous gluconate. The dosage of desferrioxamine was 92 mg/kg given intravenously on three occasions at 12-hour intervals after an initial oral dose of 5 gm. Prompt improvement in the child's clinical status was accompanied by rapid fall in serum iron concentration to normal levels and excretion of about 25 mg of iron in the urine. Further trials of desferrioxamine in the treatment of acute iron intoxication are warranted on the basis of this experience.

IRON POISONING of small children, usually from ingestion of ferrous sulfate tablets, ranks high as a cause of fatal poisoning in children in the United States. In spite of various therapeutic regimens, the mortality rate after ingestion of large doses of iron approaches 50%.[1] With increased awareness of the dangers of acute iron intoxication in children, there has been more interest in the use of iron chelating agents to lessen toxic effects of iron entering through the gastrointestinal tract. Edathamil (EDTA) has been used with apparent benefit in several cases of iron poisoning;[2] other more specific iron chelating agents, diethylenetriaminepentaacetic acid (DTPA), and ethylenediamine di (o-hydroxyphenylacetic) acid (EDDHA), have been advocated,[3, 4] though no reports of their use in iron intoxication have been made. The effectiveness of a new, more potent and specific iron chelating agent, desferrioxamine (Desferal), was tested in the treatment of a child with acute iron toxicity after ingestion of ferrous gluconate.

Report of a Case

A white female child, 14½ months of age, was admitted to St. Louis Children's Hospital on Feb 9, 1963, for treatment of acute iron toxicity from ingestion of ferrous gluconate tablets. The child had obtained a bottle of 30 to 50 ferrous gluconate tablets (0.3 gm) from an open butter dish on the bathroom sink at about 8 AM. She had eaten an unknown number of tablets when her activities were first noted at 9 AM, and the remaining pills were removed. At 9:30 AM she vomited 20 partially dissolved tablets and 30 minutes later passed seven more in several loose, bloody, diarrheal bowel movements. By 10:30 AM the child became listless, the bloody diarrhea continued, and the parents became alarmed and consulted their pediatrician. The child was taken to a local hospital where her stomach was lavaged with tap water and four more iron tablets were recovered; a 1% solution of sodium bicarbonate plus 30 ml of a combination of activated attapulgite and pectin (Quintess)® was instilled into the stomach through the tube. After an enema was given, the child was transferred to the St. Louis Children's Hospital. During the 45-minute trip she had recurrent vomiting of gray-brown, blood-tinged fluid; one more iron tablet was recovered in the vomitus—a total of 32 recovered tablets.

When first seen in the emergency room of Children's Hospital at 12:30 PM, the child was alert, irritable, and normal in color. There were stains of bloody diarrhea on her diaper. Except for a blood pressure of 90/40 mm Hg and a rapid pulse of 160 beats per minute, her physical examination was unremarkable. However, within several minutes the child became mottled, cyanotic, and obtunded with an apical heart rate of 200 beats per minute and unobtainable peripheral pulse or blood pressure. Five percent glucose in saline was administered intravenously into a scalp vein while a saphenous cutdown was performed to allow more rapid fluid replacement. Oxygen was given intermittently by face mask. Desferrioxamine-hydrochloride (800 mg in 20 ml of water) was injected intravenously after blood had been obtained for serum iron, electrolyte, and pH determinations and blood counts. Almost simultaneously, 5 gm of desferrioxamine suspended in 200 ml of isotonic saline solution were given through a nasogastric tube after aspiration of small quantities of gray-brown mucus. Within several minutes after these various forms of treatment, the child's color improved and her pulse rate slowed to 160 beats per minute though she remained unresponsive. Digitalization was begun intravenously and the patient was transferred to the Intensive Care Unit.

Initial hematological values were: hemoglobin concentration, 13.8 gm/100 cc; hematocrit, 44%; platelets, 610,000/cu mm; reticulocytes, 3.2%; white blood cell count, 39,300/cu mm; and differential count of 64% segmented neutrophils, 26% lymphocytes, 5% eosinophils, 1% band forms, and 4% monocytes. Urinalysis showed a specific gravity of 1.008, pH of 5.0, negative protein and sugar, many red and white blood cells, and a few bacteria per high power field. Serum sodium was 137 mEq/liter; chloride, 99.5 mEq/liter; potassium, 3.9 mEq/liter; carbon dioxide, 15.6 mEq/liter; and pH, 7.26. Serum iron initially was 2,550 μg per 100 cc; unsaturated serum iron binding capacity was zero. Additional laboratory data were: prothrombin level, 80%; total serum bilirubin, 0.9 mg/100 cc with 0.1 mg/100 cc direct reacting bilirubin; serum albumin, 3.6 gm/100 cc; serum globulin, 2.2 gm/100 cc; serum glu-

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tamic oxalacetic transaminase, 83 units; serum glutamic pyruvic transaminase, 16 units; serum calcium, 8.6 mg/100 cc; and serum phosphorus 3.0 mg/100 cc. A urine culture showed no growth.

The child remained lethargic for several hours in an oxygen tent with persistent red watery diarrhea and the passage of red blood that clotted on the diaper. She developed a fever to 39.2 C (102.4 F) without localizing signs of infection; penicillin and streptomycin were administered with defervescence within 36 hours. Attempts at feeding small quantities of milk at hourly intervals were abandoned because of repeated vomiting. The initial intravenous infusion of dextrose in saline was followed by successive bottles of dextrose in water and subsequently Ringer's lactate solution to alleviate the metabolic acidosis. The child's hemoglobin concentration fell from 13.4 to 11.7 gm/100 cc with a corresponding fall in hematocrit. There was an associated increase in pulse rate to 204 beats per minute, return of dusky color, and unresponsiveness; whole blood (20 ml/kg) was given with marked improvement. X-ray examination of the abdomen for additional radiopaque iron tablets showed dilatation of the small bowel with liquid radiopaque material in several loops of intestine; the liver and spleen were not enlarged.

Additional desferrioxamine hydrochloride (800 mg) was given intravenously 12 and 24 hours after the initial dose. Serial measurements of serum iron, iron binding capacity, urinary iron, and urinary desferrioxamine were obtained (Tables 1 and 2). An electrocardiogram was interpreted as showing sinus tachycardia and depression of S-T segments in leads V_1 through V_4. Twenty hours after admission (24 hours after iron ingestion), the child had the first of five convulsions with cyanosis, generalized twitching, and pooling of secretions. This seizure was controlled with paraldehyde given intramuscularly and amobarbital sodium given intravenously. Further seizures 2, 5, 6, and 12 hours later were controlled each time with intravenously administered amobarbital sodium plus additional maintenance phenobarbital. Examinations during this period showed her pupils to react to light; no abnormalities of the fundi or lungs were noted. An electroencephalogram was characterized by predominant 1-4 second slow high voltage activity mixed with some fast activity; no definite interpretation was made.

Enlargement of the liver 6 cm below the costal margin was first observed though no jaundice was seen. Repetitive blood samples showed the serum electrolyte values to have returned to approximately normal levels with correction of the acidosis. After the blood transfusion the hemoglobin concentration slowly fell to a stable level between 9.5 and 10.2 gm/100 cc with a fall of white blood cell counts to a 12,000 to 19,000 range. By the third day of hospitalization the child showed marked and sustained improvement. She was removed from the oxygen tent. Clear liquid feedings were begun and rapidly changed to a normal diet. Her indwelling catheter was removed and she played happily with no residual abnormalities except for a slight left hemiparesis first noted after her series of convulsions. Mild diarrhea without evidence of pathogenic organisms on three stool cultures responded to neomycin and a combination of kaolin and pectin (Kaopectate)® treatment. The child was returned to her home ten days after admission without any residua of her iron intoxication except the slight hemiparesis. Within a month after discharge all evidence of the hemiparesis had disappeared and a radiographic examination of the upper gastrointestinal tract was entirely normal. Her blood counts showed a hemoglobin concentration of 11.3 gm/100 cc; hematocrit, 36%; white blood cell count, 11,700 with a normal differential.

Table 1.—Measurements of Serum Iron and Iron Binding Capacity During Treatment

Date	Hour of Sample	Serum Iron, µg/100 cc	UIBC* µg/100 cc	TIBC* µg/100 cc	Comments
2/ 9/63	−1	2,550	0	2,550	Pretreatment sample
	2	2,275	0	2,275	800 mg desferrioxamine given IV at hour 0
	5	139	0	139	
2/10/63	11	600	36	636	800 mg desferrioxamine given IV at hours 12 and 24
	24	183	125	308	
	34	115	284	399	
2/11/63	44	113	270	383	
2/12/63	68	120	198	318	

*UIBC and TIBC refer to unsaturated and total iron binding capacity, respectively. UIBC was measured by the Ventura method[19]; serum iron was determined by a modified digestion technique.

Table 2.—Urinary Excretion of Iron and Desferrioxamine During Treatment

Date	Period of Sample	Urine Volume, ml	Urine pH	Urinary Iron: Conc, mg/100 cc	Urinary Iron: Total, mg	Urinary Desferrioxamine Conc, mg/100 cc
	Initial sample*	19.5	5.2	0.71	0.01	....
2/ 9/63	20 min to 2 hr	14	4.6	8.27	1.16	150
	2 to 5 hr	38	5.3	9.25	3.42	6.0
	5 to 6 hr	90	5.8	8.92	8.02	0
	6 to 11 hr	25	6.0	3.59	0.89	8.0
2/10/63	11 to 19 hr	105	6.2	3.39	3.56	48
	19 to 21 hr	43	5.8	4.10	1.77	23
	21 to 35 hr	134	5.6	4.37	5.87	42
2/11/63	35 to 43 hr	113	5.9	0.47	0.53	45.5
	43 to 46 hr	30	5.6	0.42	0.14	32
	Totals	611.5	....	....	25.37	....

*The initial sample was obtained by catheterization 20 minutes after the first dose of desferrioxamine had been given. Additional desferrioxamine was given at 12 and 24 hours. Urinary iron was determined by a modified digestion technique, desferrioxamine was measured colorimetrically.[20]

Comment

This child's story is in most respects typical of that found in acute iron toxicity. Characteristically, the child is a toddler, 12 to 30 months of age, who finds a box or bottle of iron tablets carelessly left within reach by his mother for whom they have been prescribed. The lure of colored tablets that look like candy leads the child to eat a variable number before his activities are halted by his parents or the onset of vomiting. Usually the amount of iron ingested is not precisely known, though fatal doses of ferrous sulfate have varied from 3 to 18 gm, and survival has been reported after doses as high as 15 gm.[1, 3]

The effects of ingesting toxic doses of iron have been divided into four phases chronologically.[1] The first phase begins with abdominal pain and vomiting within 30 to 60 minutes after the iron tablets are eaten. Partially dissolved tablets may be vomited along with brown or bloody stomach contents. Soon irritability, pallor, and drowsiness appear along with frequent black or bloody diarrhea. Symptoms of acidosis and cardiovascular collapse may become prominent; coma and death ensue within four to six hours in about 20% of children taking large doses of iron. The second phase consists of a period of improvement in response to treatment of the initial symptoms. Vomiting and diarrhea abate, the symptoms of acidosis and shock improve, and the child appears much less ill. This period, lasting 8 to 16 hours, may

herald the onset of progressive improvement. Often, however, the false security engendered by the transient improvement is rudely shattered by a third phase of progressive cardiovascular collapse, convulsions, coma, and high mortality at about 24 hours after iron ingestion. If this phase can be avoided or treated successfully, the child usually improves rapidly with few difficulties until one or two months later when the fourth phase of gastrointestinal obstruction from scarring occurs; corrective surgery may be required.[5]

Unusual in this child's case is the occurrence of severe iron poisoning due to ferrous gluconate. To our knowledge this is the first reported instance of ferrous gluconate poisoning in a child, though acute iron intoxication after ingestion of this iron compound has been recognized.[6] With only rare exceptions in recent reports, ferrous sulfate alone or in combination with other substances has been responsible for childhood iron poisoning.[1] On the basis of oral toxicity studies in experimental animals, ferrous gluconate is less toxic than ferrous sulfate at comparable doses of iron,[7-9] though the reasons for this difference are not clear. The toxic symptoms in this child were the same as have been described in ferrous sulfate poisoning and suggest the likelihood of severe toxic reactions from any dissociable iron compound that is absorbed rapidly in amounts sufficient to exceed significantly the maximum iron binding capacity of plasma transferrin. The ultimate pathogenesis of many of the symptoms of iron toxicity remains obscure despite extensive morphologic study and animal experimentation.[3,10-12]

On the basis of theoretical considerations, analogy to other iron chelating agents, and animal studies, the use of desferrioxamine in the treatment of acute oral iron toxicity has been suggested by several investigators.[13,14] This drug is a sideramine of microbial origin with a molecular weight of 561. As the soluble hydrochloride salt, it binds 9.3 mg of trivalent iron per 100 mg of chelate with an avidity comparable to that of the plasma iron binding protein, transferrin. Given by mouth, desferrioxamine is not absorbed to any significant degree; in the gut, especially at an acid pH, the drug binds inorganic iron and greatly reduces its absorption. Given intravenously, desferrioxamine combines with iron to form ferrioxamine which is to a large extent excreted in the urine, though some is metabolized in the body. Most of the reported studies of this chelate are concerned with its use in removing excess body iron in diseases of chronic iron storage. Relatively high levels of urinary iron excretion, ease of administration by the intravenous or intramuscular route, lack of clinically significant excretion of other metals, and freedom from serious toxic side effects make the use of desferrioxamine in the removal of excess body iron of considerable promise.[13-16]

The rationale for use of desferrioxamine in the treatment of acute iron intoxication of children is based on the twofold aim of: (1) binding iron circulating in plasma in excess of transferrin binding capacity to render it nontoxic while hastening its excretion in the urine; and (2) binding iron remaining in the gastrointestinal tract to prevent its absorption. Parenteral administration of the drug is used to effect the first aim; administration orally or by gastric tube is designed to achieve the second goal. As with use of other iron-chelating agents such as EDTA, DTPA, and EDDHA, desferrioxamine is but an adjunct to various supportive measures designed to combat symptoms of iron toxicity.

The effectiveness of desferrioxamine in the treatment of the child described in this communication is difficult to evaluate in terms of survival or effects on clinical manifestations. Criteria of drug effects that are more easily analyzed are the changes in serum iron levels and the amount of urinary iron excretion. The fall in serum iron concentration from 2,550μg/100 cc to 139μg/100 cc within five hours after intravenously administered desferrioxamine is much more rapid than has been reported in patients receiving EDTA treatment or in patients receiving no chelating agents.[2] Further evidence of efficient removal of excess iron is the reappearance of small amounts of unsaturated transferrin at 11 hours, despite high circulating serum iron levels (due in part to circulating ferrioxamine). Subsequent serum iron and unsaturated iron binding capacity values remained within the normal range. Likewise, excretion of 25.4 mg of iron in the urine during the first 43 hours of treatment is almost five times the maximum urinary iron excretion reported during a roughly comparable period after repeated EDTA infusions in a patient with an initial serum iron value of 6,260μg/100 cc.[17] No estimate could be made of the amount or sites of distribution of iron that was presumably absorbed in excess of that recovered in the urine. Only normal numbers of hemosiderin granules were observed in the reticuloendothelial cells of a bone marrow aspirate.

The value of desferrioxamine given by gastric tube to this child to prevent further iron absorption cannot be measured. It is possible that the large doses of the drug accentuated the diarrhea due to intestinal irritation, as has been reported.[16] However, the diarrhea was initiated by the iron before desferrioxamine was given; more rapid expulsion of iron bound to the desferrioxamine in the gut may have had a net beneficial effect. Theoretically, desferrioxamine should be of more value than EDTA when given by the oral route, since absorption of iron initially bound to EDTA has been shown to occur,[18] while we have preliminary evidence to suggest that radioiron bound to desferrioxamine given by mouth is not absorbed to any significant extent. Oral administration of desferri-

oxamine soon after ingestion of toxic amounts of iron would seem desirable to minimize iron absorption.

The repeated doses of desferrioxamine (92 mg/kg) given intravenously to this child are entirely empirical. Smaller concentrations of chelating agent might have been effective, though during the initial 12 hours after the first dose only small amounts of free desferrioxamine were excreted in the urine. Successively increasing amounts of iron-free drug appeared after later doses. No harmful side effects were recognized with this dosage schedule, and since data are not available on which to determine the excessive amounts of iron in the body and from this figure to calculate the required dose of chelate, it would seem justifiable to give an excess of the chelating substance.

In combating iron toxicity in children the most important measure is its prevention. This can be accomplished by warning mothers to whom iron tablets are given to keep them out of reach of young children, dispensing iron in bottles with "childproof" closures, and labeling containers with a suitable warning. When these measures fail and a child swallows a toxic dose of iron, a rational plan of treatment for acute oral iron intoxication based on a synthesis of our experience and that of others can be outlined as follows:

1. Rid the stomach of its contents. Induce emesis, lavage the stomach with a large-bore tube to remove undissolved iron tablets. Instill 5 gm of desferrioxamine in aqueous solution or, if this is not immediately available, use a 1% solution of sodium biscarbonate to bind residual iron in a poorly absorbable form. Follow the gastric lavage with an enema to remove iron from the lower bowel. If possible, obtain radiographic confirmation of the success of measures used to remove radiopaque iron from the gastrointestinal tract.

2. Institute measures to combat peripheral vascular collapse. Early intravenous replacement of body fluids and electrolytes using isotonic saline, Ringer's-lactate, plasma, dextran, or whole blood may be needed to treat the hemoconcentration and shock. Injection of an isotonic solution of desferrioxamine hydrochloride (1 gm in 25 ml of water) intravenously is warranted to bind iron circulating in excess of the transferrin binding capacity and hasten its excretion. Calcium-disodium-EDTA (80 mg/kg/24 hours) or calcium-DTPA (20 mg/kg repeated in 4 hours) may be substituted for the desferrioxamine. Repeated doses of chelating agents for 24 to 48 hours are often necessary.

3. Additional measures, often necessary, are treatment of metabolic acidosis with appropriate solutions of sodium bicarbonate. Oxygen treatment and vasopressor agents may help in combating shock. Amobarbital (Amytal), phenobarbital, paraldehyde, or diphenylhydantoin (Dilantin) may be required to control convulsions. Prophylactic antibiotics seem of value when vomiting and aspiration are severe in semicomatose patients.

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The desferrioxamine used in this study was supplied as Desferal by Ciba Pharmaceutical Company, Division of Ciba Corporation, Summit, NJ.

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UNDER THE CHARGE OF

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A REVIEW OF THE TOXICITY OF IRON COMPOUNDS

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IN recent years a number of reports have appeared on the poisoning of young children by ferrous sulfate tablets. Iron salts have been freely used as medicaments for thousands of years so that the physician ordinarily is quite unconcerned about any toxic potentialities, particularly when the compound is to be given orally. Since this lack of fear of toxicity seemed inconsistent with the clinical toxicological reports, it became desirable to review the literature on comparative toxicity of various iron salts in experimental animals and in accidental poisonings in patients. The literature survey also indicated the desirability of a new direct experimental comparison under critical conditions of the tolerance of ferrous gluconate and ferrous sulfate. This study has been carried out and is reported elsewhere[28].

History of Iron Therapy. The origin of iron therapy is obscure in the dimness of prehistoric medical experience. It is known to have been employed by the ancient Hindus, Egyptians and Greeks. Iron (apparently as the sulfate) was one of the few inorganic medicines described in the old Egyptian pharmacopoeias. Similarly, in an attempt to bestow the strength of iron upon a patient, Greek physicians administered the metal as a cure for

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weakness (which is one of the prominent symptoms of anemia). Water in which red hot iron had been quenched or in which swords had rusted was frequently the medicinal form of iron[30]. Hippocrates also recommended it for both diarrhea and constipation.

In the seventeenth century Sydenham wrote of the treatment of chlorosis by iron[32]:

"To the worn out or languid blood it gives a spur or fillip whereby the animal spirits which before lay prostrate and sunken under their own weight are raised and excited. Clear proof of this is found in the effect of steel in chlorosis. The pulse gains strength, the face (no longer pale and death-like) a fresh ruddy color."

Iron was first shown to be present in the blood in the eighteenth century and Menghini[30] demonstrated that iron in the blood could be increased by feeding foods rich in that substance. Still another significant advance, in the same century, was William Cullen's prophetic warning that the good effects of iron were often missed because of too small doses.

But iron therapy reached its golden age with Pierre Blaud's introduction of his famous pill in 1831[30]. For most of the remainder of the century eulogies proclaiming iron first among all therapeutic agents were common. Then, Bunge, Quincke, and others, finally convinced physicians that inorganic iron was hardly absorbed at all. This, coupled with the unsatisfactory results of injudicious use of iron in all types of anemia, led at last to the "Dark Ages" of iron therapy[21]. Ferrous sulfate, for instance, was not even considered for internal use in the United States Dispensatory of 1918. It was only after the first quarter of the present century that the value of large doses of iron, where iron was needed, was again recognized[29,70].

The frequency of poisoning by iron appears to be a direct function of the fashion in iron therapy in any given period. Opinions on the noxious effects of this substance have varied widely. Sydenham maintained that "iron may be given in the largest doses without inconvenience." However, in 1851, Orfila[54] pleaded, because of the increasing number of accidental and homicidal poisonings from iron salts, for recognition of the toxicity of ferrous sulfate which he and Smith had demonstrated 36 years earlier in *1815*. The law courts of France[7,8,14,44,79] and Italy[28,59] in the middle of the nineteenth century, confused by the divided opinions on the toxicity of iron salts, turned to medical men who carried out animal experiments. Based on these results, iron salts were ruled "poisons" in the legal sense and their administration for felonious purpose constituted "attempted premeditated murder"[59,78].

However, after the turn of the present century, the dispute as to the absorbability of inorganic iron led to the disappearance of iron preparations from the family medicine chest, and iron poisonings vanished. Then observe the trend in thinking on iron as knowledge of the older clinical and experimental reports dimmed and was lost:

1904: "Sufficient evidence exists that ferrous sulfate and ferric chloride have toxic properties"[55].

1928: "Fatal poisoning in man is exceptional"[69].

1934: "Cases of poisoning due to ingestion of iron are extremely rare"[25].

1941: "General intoxication from orally administered iron therapy is unknown"[30].

With the discovery that orally administered iron is utilized in the body and with the gradual acceptance of its safety, particularly when compared to

reactions after parenteral administration[39,43,60], ferrous iron has returned to prominence. In fact so popular is this remedy that five hospitals serving 400,000 people dispensed half a million iron pills in a recent period of 6 months[21].

Since ferrous sulfate tablets are often brightly colored and sugar- or chocolate-coated, they may have a tempting appeal for small children, and hence lead to accidental poisoning[16,22,76]. When reports of such cases began to appear, interest in the toxicology of iron quickened. However, the older literature seems largely to have escaped attention. Thus, Somers[71] reported that "examination of the literature failed to reveal earlier reports of ill effects from orally administered iron compounds. Further . . . we have been unable to find any account of pharmacological investigation into the action of iron given by mouth."

Attention was drawn to this problem in 1952 by the editors of the *Journal of Pediatrics*[19] as follows:

"It is puzzling to understand why medicinal iron preparations, which have been used for generations and which have been looked upon as almost innocuous in overdosage according to medical texts, should first be reported in the last few years as a cause of severe and fatal accidental poisoning in young children. It is obvious that the potential dangers of medicinal iron as a cause of accidental poisoning should be better known to physicians and the public. . . ."

In view of the conflicting evidence and, more important, the increasing frequency of fatalities following the oral ingestion of iron salts, particularly in infants and children, it has become desirable to take a more extensive look at the literature on the toxicity of the iron preparations available for medicinal use. There are summarized below the results of this search of the literature.

Toxicology of Iron Salts in Animals. Estimates of the median lethal dose for several iron preparations by various routes of administration in experimental animals are summarized in Tables 1 to 4. An attempt has been made to express the data in terms of the median lethal dose as mg./kg., both as the salt and its equivalent in terms of ionic iron. The source of the data is indicated in each instance by the reference.

Particularly striking is the fact that relatively few attempts have been made to establish the acute toxicity of these preparations in experimental animals with any degree of precision. In many instances considerable difficulty was encountered by us in attempting, from the published data, to establish the form of the preparation used, the manner in which it was given, duration of the observations, and number of animals employed. Thus, finding a means of expressing the data in standard terminology was a definite problem. From Tables 1 and 4, it becomes possible to arrange the compounds in order of increasing oral toxicity in animals, as follows:

Compound	*Species*	*Estimated Oral LD₅₀ mg./kg.*
Ferrous gluconate	mouse	6600
	guinea pig	2100
	rabbit	3500
Ferric ammonium citrate	mouse	5000
	guinea pig	1750
	rabbit	2800
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	mouse	4500
	guinea pig	1500
	rabbit	3000
	cat	>500
	dog	800
Ferric chloride	mouse	1500
	guinea pig	600
	rabbit	1200
Ferrous chloride	rat	600
	rabbit	1000

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	Species	Estimated Oral LD_{50} mg./kg.
ate	mouse	6600
	guinea pig	2100
	rabbit	3500
m citrate	mouse	5000
	guinea pig	1750
	rabbit	2800
	mouse	4500
O)	guinea pig	1500
	rabbit	3000
	cat	>500
	dog	800
	mouse	1500
	guinea pig	600
	rabbit	1200
	rat	600
	rabbit	1000

TABLE 1.—TOXICITY OF FERROUS SULFATE (ORAL ADMINISTRATION)

pecies	Dose* As Salt	Dose* As Fe^{++}	LD_{50} mg./kg. As Salt	LD_{50} mg./kg. As Fe^{++}	Comments	Ref.
se	..	..	4500	900	$FeSO_4$ crystalline	71
	..	..	4100	1000	$FeSO_4$ as "Fersolate"	71
	..	..	..	710		13
	..	29.4 mg.	..	..	Fatal. Animal wts. 35 to 45 gm.	73
	..	73.6 mg.	..	..	1/3 died. Animal wts. 100 to 200 gm.	73
ea pig	400 mg.	..	..	..	Fatal 18½ hrs. Fe as "Fersolate"	25
	200 mg.	..	..	..	Fatal in ½ hr.	25
	400 mg.	..	..	..	Survived } Fatal dose equals	25
	600 mg.	..	..	..	Fatal } 65 mg./64 gm.	25
	800 mg.	..	..	..	Fatal	25
	..	..	1500	300	$FeSO_4$ crystalline	71
bit	..	..	1250	300	$FeSO_4$ as "Fersolate"	71
	..	..	3000	600	$FeSO_4$ crystalline	71
	..	..	3000	720	$FeSO_4$ "Fersolate"	71
	3000 mg./kg.	..	..	..	With 3 gm. $NaHCO_3$. Survived	71
	3000 mg.	..	..	..	Fatal	79
	..	368 mg./kg.	..	..	Ill but survived	73
	..	736 mg./kg.	..	..	Fatal	73
	1000 mg.	..	..	..	In 30 to 50 gm. corn meal. No ill effects	28
	4327 mg./kg.	..	..	..	Fatal 3 to 4 hours	28
	1869 mg./kg.	..	..	..	Fatal < 1 hour	28
	769 mg./kg.	..	..	..	Fatal 1½ hours	28
	540 mg./kg.	..	..	..	Ill but survived	28
	1000 mg.	240 mg.	..	..	5 "Fersolate" tablets. Survived	25
	2000 mg.	..	..	..	Ill but survived	69
	8000 mg.	..	..	..	Fatal in 26 hrs.	54
	930 mg./kg.	..	..	..	Fed in cornmeal. Ill but survived	28

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 2.—TOXICITY OF FERROUS SULFATE (INTRAVENOUS ADMINISTRATION)

Species	Dose* As Salt	Dose* As Fe^{++}	LD_{50} mg./kg. As Salt	LD_{50} mg./kg. As Fe^{++}	Comments	Ref.
use	..	..	..	18.8		13
	..	..	..	11		51
bbit	..	30–60 mg./kg.	..	..	Fatal dose lies in this range	47
t	..	10–15 mg./kg.	..	..	Fatal in nine hours	69
	..	30–60 mg./kg.	..	..	Fatal dose lies in this range	47
g	..	30 mg./kg.	..	..	Lethal dose for dog	47
	400–500 mg.	..	..	..	Ill but survived	54
	..	10 mg./kg.	..	..	No effect	74
	..	20 mg./kg.	..	..	Fatal in three hours	74
	..	70 mg./kg.	..	..	Immediate death	74

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 3.—TOXICITY OF FERROUS SULFATE (RECTAL AND TOPICAL ADMINISTRATION)

Species	Route of Adminis.	Dose* As Salt	Dose* As Fe^{++}	Tissue Irritation	Comments	Ref.
t	Rectal	..	36.8 mg.	..	Fatal in 3 hrs. }	73
		..	73.6 mg.	..	Died in ½ hr. } Wt. 100 to 200 gm.	73
bbit		..	73.6 mg.	..	Died in 4 hrs. }	73
g	Rectal	..	368 mg./kg.	..	Fatal in 6 hours	73
	Topical	8000 mg.	..	Intense	Fatal in 12 to 27 hours when applied to cellular tissue of thigh	54

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 4.—TOXICITY OF OTHER IRON SALTS

Salt	Species	Route of Admin.	Dose* As Salt	Dose* As Fe^{++}	LD_{50} mg./kg. As Salt	LD_{50} mg./kg. As Fe^{++}	Comments	Ref.
Ferrous glucon.	Mouse	Oral	..	..	6600	1100	Figures are those reported by the author who indicated iron content as 16.2/3%	
	Guinea pig	"	..	..	2100	350		
	Rabbit	"	..	..	3500	580		
Ferrous chlor.	Frog	"	..	22 mg.	..	..	Fatal in 24 hrs. Wt. 35 to 45 gm.	
	"	"	..	33 mg.	..	..	Fatal in 4 hrs.	
	Rat	"	..	14 mg.	..	..	Somewhat ill	
	"	"	..	18 mg.	..	..	Somewhat ill	Wt. 100 to 200 gm.
	"	"	..	28 mg.	..	..	1/2 died after 24 hrs.	
	"	"	..	56 mg.	..	..	Fatal 7/7 in ½ to 30 hrs.	
	Rabbit	"	..	168 mg./kg.	..	..	No effect	
	"	"	..	224 mg./kg.	..	..	2/2 no effect	
	"	"	..	252 mg./kg.	..	..	Fatal in 24 hrs.	
	"	"	..	280 mg./kg.	..	..	Fatal in 5/5 in 24 to 48 hrs. Higher doses all fatal	
Ferrous carb.	Mouse	"	..	..	31000	3800	As Blaud's Pills	
	"	"	..	..	16000	2000	" " "	
	Guinea pig	"	..	..	17800	2220	" " "	
	Rabbit	"	..	..				
Ferric chlor.	Mouse	"	..	..	1500	500		
	"	"	..	..	..	840		
	Guinea pig	"	..	..	600	200		
	Rabbit	"	..	..	1200	400		
	Dog	"	3.75–5 gm.	..	..	..	Fatal in 27 to 30 hrs.	
	"	"	2.5 gm.	..	..	..	Severely ill one week, impaired digestive process	
Ferric ammon. cit.	Mouse	"	..	..	5000	1000		
	Guinea pig	"	..	..	1750	350		
	Rabbit	"	..	..	2800	560		
Sodium ferricit.	Rat	"	..	37.2 mg./kg.	..	..	1/2 dead in 2 hrs.	
	Rabbit	"	..	186 mg./kg.	..	..	Fatal in 4 hrs.	
Ferrous chlor.	Dog	I.V.	..	10 mg./kg.	..	..	No effect	
	"	I.V.	..	30 mg./kg.	..	..	Fatal in 8 hrs.	
Ferric chlor.	Mouse	I.V.	..	..	..	18.5		
Ferric ammon. cit.	Mouse	I.V.	..	..	..	16.5		
Sodium ferricit.	Frog	S.C.	..	5–10 mg.	..	..	Produced paralysis	
	Rabbit	I.V.	..	25 mg./kg.	..	..	Average lethal dose	
	Cat	I.V.	..	30-60 mg./kg.	..	..	Lethal. No symptoms for 3 days	
	Dog	I.V.	..	20-50 mg./kg.	..	..	Lethal	
Ferrous bicarb.	Dog	I.V.	..	5 mg./kg.	..	..	No effect	
	"	I.V.	..	10 mg./kg.	..	..	Fatal in 3 hrs.	
Ferric tartrate	Mouse	I.V.	..	..	..	16.5		
Ferrous chlor.	Rat	Rectal	..	28 mg.	..	..	Fatal in 48 hrs.	
	"	"	..	56 mg.	..	..	Fatal in 5 min.	
	Rabbit	"	..	280 mg./kg.	..	..	Fatal in 1/2 in 5 hours	
Sodium ferricit.	Rat	"	..	37 mg./kg.	..	..	Fatal in 5 hrs.	
	Rabbit	"	..	186 mg./kg.	..	..	Fatal in 4 hrs.	

* Where dose is given only as the salt the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

I.V. = intravenous S.C. = subcutaneous

The intravenous to
little more difficult
largely to the pauci
available data indica
striking clarity: these
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ministration. The int
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be approximately 70
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ferrous sulfate. An i
of 30 mg./kg. of iron
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Following oral a
toxic doses of ferrou
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the animal becomes
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into complete prostrat
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rapid. Most of the dea
within 2 to 6 hours,
terminal convulsive e
of respiration precede
Those animals which s
show evidence of an
the day following m
survivors also exhibit
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during the first 2 or 3 d
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nd dog, appear to b
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Inspection of the vis
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pon the dose and con
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petechial hemorrha

OTHER IRON SALTS

g. kg.		Comments
1= Fe^{++}		
1100		Figures are those reported by the aut
350		or who indicated iron content as
580		2/3
--		Fatal in 24 hrs. Wt. 35 to 45 gm.
--		Fatal in 4 hrs.
--		Somewhat ill
--		Somewhat ill
--		1/2 died after 24 hrs. Wt. 100 t
--		Fatal 7/7 in ½ to 30 hrs. 200 gm.
--		No effect
--		2/2 no effect
-		Fatal in 24 hrs.
-		Fatal in 5/5 in 24 to 48 hrs. Highe doses all fatal
90		As Blaud's Pills
90		" " "
20		" " "
0		
0		
0		
0		
		Fatal in 27 to 30 hrs.
		Severely ill one week, impaired diges tive process
		1/2 dead in 2 hrs.
		Fatal in 4 hrs.
		No effect
		Fatal in 8 hrs.
		roduced paralysis
		verage lethal dose
		thal. No symptoms for 3 days
		ibal
		effect
		tal in 3 hrs.
		al in 48 hrs.
		l in 5 min.
		l in 1/2 in 5 hours
		in 5 hrs.
		in 4 hrs.
		indicated the state of hydra ated.

The intravenous toxicity data are a tle more difficult to appraise due rgely to the paucity of data. The ailable data indicate one fact with riking clarity: these preparations are nsiderably more toxic by the intra- enous than by the oral route of ad- inistration. The intravenous toxicity f ferrous sulfate in mice appears to e approximately 70 mg./kg.[13]. Inter- stingly, the intravenous toxicity value or ferric chloride appears to be of an rder of magnitude similar to that for errous sulfate. An intravenous value of 30 mg./kg. of iron or approximately 107 mg./kg. of salt was reported for ferrous chloride in the dog[73].

Following oral administration of toxic doses of ferrous sulfate and related compounds in the mouse and rat, the animal becomes depressed within a few minutes. This depression deepens into complete prostration during which the respiration becomes shallow and rapid. Most of the deaths usually occur within 2 to 6 hours, following a brief terminal convulsive episode. Cessation of respiration precedes cardiac arrest. Those animals which survive invariably show evidence of an intense diarrhea the day following medication. These survivors also exhibit a decreased interest in food for a day or so and frequently there are delayed deaths during the first 2 or 3 days. Toxic symptoms in higher species, such as the cat and dog, appear to be similar except that copious vomiting is produced in contrast to the lower rodent species, where this protective mechanism is absent.

Inspection of the viscera immediately following death reveals the presence of mild to severe congestion of the gastric mucosa even to the point of fresh blood in the stomach, depending upon the dose and concentration of the preparation administered. Hyperemic to petechial hemorrhagic areas may be found in the small intestine. The liver usually shows marked congestion and several to many petechial hemorrhagic areas are usually seen in the lungs. Tissue changes present at death occurring several days after oral medication include marked erosion of the gastric mucosa with fibrotic changes particularly in the greater curvature and antrum, and congestion in the liver, lungs and kidney.

Recently, Nissim[52] called attention to the capillary damaging and anticoagulant effects of various iron preparations and the striking agreement with the incidence of extensive hemorrhages in the lungs with these preparations. Interestingly enough, some 90 years ago, Tourdes[79] observed a "thinning of the blood" in experimental animals suffering from iron intoxication and had suggested that ferrous sulfate may inhibit the coagulation of blood.

CHRONIC TOXICITY OF IRON COMPOUNDS. Studies by Hendrysch and Klimesch[35], using ferrous carbonate, ferrous chloride, and sodium ferricitrate intramuscularly or subcutaneously in rabbits and dogs, showed that administration of small amounts of these iron compounds over periods up to 4 months produces a chronic and sometimes fatal poisoning. These authors concluded that the differential toxicity of iron salts is not based strictly on iron content. Hoff[36] administered small daily doses of ferric chloride (about 300 mg. of iron or about 870 mg. of anhydrous ferric chloride) to a dog in which the liver was by-passed by means of an Eck fistula. "Chronic cerebral intoxication" was reported.

Clinical Toxicity. Ferrous sulfate is the causative agent in the majority of iron poisonings, but fatal ingestion of ferrous chloride, ferric chloride, and ferric ammonium citrate has been reported. In every case, nineteenth century and contemporary, the clinical

aspects have been surprisingly similar. Initially there appear nausea and some vomiting, progressing to severe gastroenteritis with hematemesis, abdominal pain, and diarrhea. Lassitude is followed closely by development of marked shock, usually 4 to 6 hours after ingestion. If the patient survives this collapse, there generally ensues a period of considerable clinical improvement. A second crisis occurs 20 to 50 hours after ingestion of the iron preparation; and if this latter stage of shock, arising from gastric mucosal corrosion, does not terminate fatally, recovery is usually ensured. Hematochezia, convulsions, and motor disturbances are seen occasionally[1,19,31,64,75,79]. Postmortem findings include necrosis of the gastric and intestinal mucosa and congestion or necrosis of the liver. In addition, lung and kidney congestion are frequently observed. Fatal outcome following overdosage with iron varies widely, not only with dose, but also with age, physical condition, and individual susceptibility.

CASE REPORTS ON OVERDOSAGE WITH ORALLY INGESTED IRON PREPARATIONS

A. *Ferrous Sulfate (36.76% iron in anhydrous salt, 20.09% in USP crystalline).* Ferrous sulfate has been the toxic agent in nearly all the reported poisonings, accidental and homicidal. Of the 63 cases with this salt, 23 (two adults and 21 children) ended fatally. In many of the recent instances, the source of iron was "Fersolate," a British proprietary preparation consisting of 200 mg. (3 gr.) of $FeSO_4$, 2.6 mg. (1/25 gr.) of $CuSO_4$, and 2.6 mg. (1/25 gr.) $MnSO_4$ per sugar coated tablet. As few as 15 to 16 of these tablets in a single dose have proved fatal to a 19-month-old child, and 8 are reported to have produced a severe reaction in a child of 2 years. It should be noted that laboratory tests indicate that neither the manganese nor the copper sulfate present contribute materially to the toxic action[25,71]. Obstruction of the stomach occurred in 7 cases. Two instances are considered in detail, one a 3-year-old boy who had ingested about 67 ferrous sulfate tablets[10] and the second, a 17-month-old boy who swallowed 6 to 12 "Fersolate" tablets[62]. Each patient exhibited typical symptoms of ferrous sulfate poisoning so that gastric lavage was performed and anti-shock treatment administered. After several days, both had improved and were vomiting only occasionally. About 3½ weeks after ingestion, emesis increased in frequency and severity. Radiograms made 4 hours after a barium meal showed no barium had left the stomach of either child. In the first case, the stomach was empty 24 hours later, but in the second, only a small amount of barium was observed in the transverse colon after approximately 20 hours. Both children were clinically worse and surgery seemed the best course. Upon operation, thickening and stenosis of the pylorus were found, which were more severe in the case of the younger child. The first patient made a satisfactory recovery, but the second died of acute suppurative peritonitis following the operation.

In both animals and humans who have died after overdoses of iron, hemorrhagic gastritis with edema has been observed in postmortem examination. Both Crosskey and Ross felt that fibrous contracture of the pyloric antrum and pyloric stenosis probably resulted from this persistent intense gastritis.

A summary of fatal cases appears in Table 5 and of nonfatal, in Table 6.

It should be pointed out that in many cases authors have not identified the preparation nor indicated the state of hydration of the ferrous sulfate. Different manufacturers declare in terms of the anhydrous, exsiccated or

U.S.P. (crystalli
no indication at
dration. General
the 0.2 gm. tabl
rous sulfate U.S.

TABL

No.	*Year*	
1	1850	Chi
2	1851	Ad
3	1851	4 y
4	1851	10
5	1888	5 y
6	1947	3½
7	1947	16
8	1947	12
9	1948	26
10	1949	11
11	1950	17
12	1951	12
13	1951	19
14	1951	18
15	1951	14
16	1952	26
17	1952	19
18	1952	21
19	1952	17
20	1952	2 y
21	1953	29
22	1954	20
23	1954	21

NOTE: Cas
to 15 were pr
* Based on
line ferrous s

it contribute materially to the action[26,71]. Obstruction of the ch occurred in 7 cases. Two in- s are considered in detail, one ear-old boy who had ingested 67 ferrous sulfate tablets[10] and econd, a 17-month-old boy who wed 6 to 12 "Fersolate" tablets[62]. patient exhibited typical symp- of ferrous sulfate poisoning so astric lavage was performed and ock treatment administered. several days, both had improved ere vomiting only occasionally. 3½ weeks after ingestion, emesis sed in frequency and severity. grams made 4 hours after a bar- eal showed no barium had left mach of either child. In the first he stomach was empty 24 hours but in the second, only a small it of barium was observed in the erse colon after approximately urs. Both children were clinically and surgery seemed the best . Up operation, thickening and s of the pylorus were found, were more severe in the case of unger child. The first patient a satisfactory recovery, but the died of acute suppurative peri- following the operation.

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mmary of fatal cases appears in 5 and of nonfatal, in Table 6. ould be pointed out that in ases authors have not identified paration nor indicated the state ration of the ferrous sulfate. nt manufacturers declare in f the anhydrous, exsiccated or U.S.P. (crystalline) salt; some make no indication at all of the state of hydration. Generally, one can assume that the 0.2 gm. tablets are exsiccated ferrous sulfate U.S.P. (approximately 30% iron) and the 0.3 gm. ones are U.S.P. crystalline ferrous sulfate (approximately 20% iron), although this is not invariably the case. Further confusion exists among different official prepara-

TABLE 5.—SUMMARY OF DEATHS DUE TO FERROUS SULFATE

No.	*Year*	*Age*	*Sex*	*Approximate Dose of $FeSO_4$**	*Time of Death after Ingestion*	*Comments*	*Ref.*
1	1850	Child	?	? plus alum	?	Murder. Sentenced to 10 years enforced labor	14
2	1851	Adult	M	? in beef broth	36 hrs.	Murder. Wife condemned to death	8
3	1851	4 yrs.	?	?	?	Murder	54
4	1851	10 mo.	F	50 gm.	36 hrs.	Murder	54, 78
5	1888	5 yrs.	M	648 mg.	24 hrs.	Accident. Intended as an anthelmintic	24
6	1947	3½ yrs.	M	10 gm.	53 hrs.	Accidentally ingested 50 Fersolate tablets	25
7	1947	16 mo.	F	5.2 gm.	21 hrs.	Accident. Source was 26 Ferosolate tablets	76
8	1947	12 mo.	M	6–7 gm.	30 hrs.	Accident. 30 to 35 Fersolate tablets. Treated for shock and aspiration pneumonia	25
9	1948	26 yrs.	M	115 gm.	3 hrs.	About ¼ lb. of U.S.P. ferrous sulphate	27
10	1949	11 mo.	F	?	39 hrs.	Accidentally ingested unknown quantity of Fersolate tablets	57
11	1950	17 mo.	F	6 gm.	11 hrs.	Accident. No more than 20 × 0.3 gm. $FeSO_4$ tablets. Methylene blue gave temporary improvement.	67
12	1951	12 mo.	M	?	4½ hrs.	Accident. Unknown number of $FeSO_4$ tablets. Only medical treatment consisted of castor oil	72
13	1951	19 mo.	F	3.0–3.2 gm.	4¾ hrs.	Accident. 15 to 16 $FeSO_4$ tablets. Two hospitals refused admission. Doctor prescribed orange juice	72
14	1951	18 mo.	M	8.8 gm.	5½ hrs.	Accident. 44 $FeSO_4$ tablets. Stomach lavaged. Restoratives given	72
15	1951	14 mo.	F	8 gm.	20 to 24 hrs.	Accident. 44 $FeSO_4$ tablets. Returned 4. Doctor felt no danger, prescribed castor oil and kaolin	72
16	1952	26 mo.	F	9 to 12 gm.	4½ hrs.	Accident. 30 to 40 × 0.3 gm. chocolate coated tablets. Gastric lavage plus supportive therapy	12
17	1952	19 mo.	M	?	40 hrs.	Accident. Unknown number enteric coated 0.2 gm. tablets. Gastric lavage, supportive therapy, antibiotics, BAL without improvement	75
18	1952	21 mo.	M	8.2 gm.	4 hrs.	Accident. About 41 Fersolate tablets. Gastric lavage with sodium bicarbonate	66
19	1952	17 mo.	M	?	?	Accident. Unknown quantity of tablets	80
20	1952	2 yrs.	M	13.8 gm.	7 hrs.	Accident. About 43 × 0.32 gm. $FeSO_4$ tablets	80
21	1953	29 mo.	M	22.5 gm.	4½ hrs.	Accident. 75 × 0.3 gm. tablets. Gastric lavage	4
22	1954	20 mo.	F	10.2 to 14.2 gm.	20⅔ hrs.	Accident. 34 to 44 × 0.3 gm. enteric coated $FeSO_4$. Supportive therapy	9
23	1954	21 mo.	F	?	48 hrs.	Accident. ? × $FeSO_4$ exsic. 0.162 gm. + liver conc. NF. Supportive therapy	11

NOTE: Cases 1 to 5 were probably $FeSO_4 \cdot 7H_2O$ but authors do not so indicate. Cases 12 to 15 were probably Fersolate.

* Based on each author's report. No attempt has been made to convert U.S.P. crystalline ferrous sulfate ($FeSO_4 \cdot 7H_2O$). [See text.]

TABLE 6.—NONFATAL POISONING FROM FERROUS SULFATE

No.	*Year*	*Age*	*Sex*	*Approximate Dose of $FeSO_4$**	*Length of Convalescence*	*Comments*	*Ref.*
1	1850	22 yrs.	F	?	?	Attempted murder. Husband sentenced to 5 yrs. Nearly fatal. Commercial green vitriol	7
2	1859	36 yrs.	M	? gm. in wine	3 days	Attempted murder. Commercial ferrous sulfate. Seriously ill	78
3	1881	17 yrs.	M	?	..	Attempted murder. Commercial ferrous sulfate. Small amount of cornmeal. Slightly ill.	28
4	1881	12 yrs.	F	?	Several days	Sister of Case 3. Poisoned on same occasion	28
5	1881	45 yrs.	F	?	2 weeks	Mother of Cases 3 and 4. Poisoned on same occasion	28
6	1881	70 yrs.	M	?	2 days	Father of Cases 3 and 4. Poisoned on same occasion	28
7	1883	40 yrs.	F	56 gm.	3 mo.	Attempted suicide. Stormy course for more than 2 mos.	34
8	1934	Child	F	28 gm.	?	No details given	25, 67, 68
9	1936	30 yrs.	M	32.4 gm. in 26 days	?	3 × 0.375 gm. per day. Anemia therapy. Epileptiform seizures. Patient weighed 86.5 lbs.	50
10	1936	Adult	F	24.75 gm. in 33 days	?	0.75 gm./day. Anemia therapy. Epileptiform seizures	50
11	1947	2 yrs.	M	1.6 gm.	15 days	Accident. 10 Fersolate tablets. Returned 2. Emetics and supportive therapy	76
12	1949	16 mo.	M	6 gm.	1 week	Accident. About 50 Fersolate tablets. Returned 20. Gastric lavage, supportive therapy and BAL	63
13	1950	4½ yrs.	F	0.8 gm.	..	Accident. 24 Fersolate tablets. Returned 20. Received 0.15 gm. $NaHCO_3$ every 4 hrs.	77
14	1950	19 mo.	M	2 gm.	..	Accident. About 10 Fersolate tablets. Gastric lavage with $NaHCO_3$; BAL	77
15	1950	2½ yrs.	M	2 to 4 gm.	..	Accident. 10 to 20 Fersolate tablets. Given syrup of figs	77
16	1951	14 mo.	?	4 gm.	..	Accident. 19 to 20 $FeSO_4$ tablets	6
17	1951	2½ yrs.	F	?	3 days	Accident. About 60 $FeSO_4$ tablets but returned "nearly all"	72
18	1951	21 mo.	F	10.8 gm.	26 days	Accident. About 75 $FeSO_4$ tablets but returned 21. Gastric lavage	72
19	1951	23 mo.	M	6.5 gm.	3 weeks	Accident. 16 $FeSO_4$ tablets and 10 iron "plastules." Returned 4 tablets and partly dissolved "plastules"	72
20	1951	11 mo.	M	1.4 to 1.8 gm.	3 days	Accident. 13 $FeSO_4$ tablets but returned pieces = to 4 to 6 tablets. Gastric lavage	72
21	1951	20 mo.	M	0.6 gm.	3 hrs.	Accident. About 5 $FeSO_4$ tablets but returned 2. Ill enough to hospitalize	72
22	1951	30 mo.	F	15 gm.	11 days	Accident. Believe about 75 × 0.2 gm. $FeSO_4$ tablets. Gastric lavage. $NaHCO_3$. Penicillin	49
23	1952	15 mo.	F	4.5 to 6 gm.	54 hrs.	Accident. 15 to 20 × 0.3 gm. $FeSO_4$ tablets. Tablet fragments returned	12
24	1952	18 mo.	M	4.5 gm.	7 days	Accident. 15 × 0.3 gm. $FeSO_4$ tablets. Gastric lavage, plasma, penicillin	12
25	1952	3 yrs.	M	?	2½ mo.	Accident. 67 × ? gm. $FeSO_4$. Gastric lavage, nikethamide, and methionine. Pyloric stenosis necessitated surgery	10
26	1952	19 mo.	F	?	1 week	Accident. 10 × ? $FeSO_4$ tablets. Gastric lavage and supportive therapy	40

TABLE 6.—NONF

No.	*Year*	*Age*
27	1953	17 mo.
28	1954	14 mo.
29	1954	21 mo.
30	1954	2 yrs.
31	1954	26 mo.
32	1954	16 mo.
33	1954	15 mo.
34	1954	13 mo.
35	1954	17 mo.
36	1954	13 mo.

NOTE: No details a
solate.
* Based on each au
line Ferrous Sulfate (

tions of the exsiccate
material containing
anhydrous salt, FeSO
less than 77%, and o
Norway 80.5 to 85%.
stance, declares 0.2
(at least 77% anhydr
to about 29% ferrous i
cation from the n
search laboratories
content as 24%[71]. Th
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line ferrous sulfate
content frequently
mined with any acc

In some instances
13, 15), the children

ROUS SULFATE

Comments	Ref.
pted murder. Husband sen-ed to 5 yrs. Nearly fatal. Com-cial green vitriol	7
pted murder. Commercial fer-s sulfate. Seriously ill	78
pted murder. Commercial fer-s sulfate. Small amount of corn-l. Slightly ill.	28
of Case 3. Poisoned on same oc-on	28
er of Cases 3 and 4. Poisoned on ne occasion	28
r of Cases 3 and 4. Poisoned on ne occasion	28
pted suicide. Stormy course for re than 2 mos.	34
tails given	25, 67, 68
0.375 gm. per day. Anemia ther-. Epileptiform seizures. Patient ghed 86.5 lbs.	50
cm./day. Anemia therapy. Epi-tiform seizures	50
ient. 10 Fersolate tablets. Re-rned 2. Emetics and supportive rapy	76
ient. [illegible]ut 50 Fersolate tablets. [illegible]turne[illegible]. Gastric lavage, sup-rtive therapy and BAL	63
ient. 24 Fersolate tablets. Re-rned 20. Received 0.15 gm. Na-CO$_3$ every 4 hrs.	77
dent. About 10 Fersolate tablets. stric lavage with NaHCO$_3$; BAL	77
dent. 10 to 20 Fersolate tablets. ven syrup of figs	77
dent. 19 to 20 FeSO$_4$ tablets	6
dent. About 60 FeSO$_4$ tablets but turned "nearly all"	72
dent. About 75 FeSO$_4$ tablets but turned 21. Gastric lavage	72
dent. 16 FeSO$_4$ tablets and 10 iron plastules." Returned 4 tablets and rtly dissolved "plastules"	72
dent. 13 FeSO$_4$ tablets but re-rned pieces = to 4 to 6 tablets. astric lavage	72
dent. About 5 FeSO$_4$ tablets but turned 2. Ill enough to hospitalize	72
dent. Believe about 75 × 0.2 gm. eSO$_4$ tablets. Gastric lavage. Na-CO$_3$. Penicillin	49
dent. 15 to 20 × 0.3 gm. FeSO$_4$ blets. Tablet fragments returned	12
dent. 15 × 0.3 gm. FeSO$_4$ tablets. astric lavage, plasma, penicillin	12
dent. 67 × ? gm. FeSO$_4$. Gastric avage, nikethamide, and meth-onine. Pyloric stenosis necessitated urgery	10
dent. 10 × ? FeSO$_4$ tablets. Gas-ric lavage and supportive therapy	40

TABLE 6.—NONFATAL POISONING FROM FERROUS SULFATE—(*Continued*)

No.	Year	Age	Sex	Approximate Dose of $FeSO_4$*	Length of Convalescence	Comments	Ref.
27	1953	17 mo.	M	1.2 to 2.4 gm.	Died of peritonitis following surgery	Accident. 6 to 12 Fersolate tablets. Gastric lavage and antishock treatment. Pyloric stenosis necessitated surgery twice	62
28	1954	14 mo.	F	15 to 22.5 gm.	10 days	Accident. 50 to 75 × 5 gr. $FeSO_4$ tablets. Gastric lavage and BAL	65
29	1954	21 mo.	F	?	8 weeks	Accident. Unknown number of Fersolate. Pyloric stenosis requiring surgery	81
30	1954	2 yrs.	M	8 gm.	1 mo.	Accident. 40 Fersolate. $NaHCO_3$ lavage returned broken tablets. Pyloric stenosis treated surgically	81
31	1954	26 mo.	F	13 gm.	6 days	Accident, 65 capsules × 0.2 gm. $FeSO_4$, 3.25 mg. molybdenum oxide. Gastric lavage. I.V. $NaHCO_3$ and BAL	3
32	1954	16 mo.	F	?	8 weeks	Accident. ? × Ferrous Sulfate tablets. Vomited 20. $NaHCO_3$ lavage. Pyloric stenosis required surgery	23
33	1954	15 mo.	F	2.4 gm.	4 days	Accident. 8 × 0.3 gm. $FeSO_4$ tablets. enteric-coated	9
34	1954	18 mo.	F	3.8 to 5.7 gm.	8 days	Accident. 20 to 30 × 0.19 gm. $FeSO_4$ tablets. $NaHCO_3$ lavage. Supportive therapy	42
35	1954	17 mo.	F	2 to 3 gm.	3½ mo.	Accident. 10 to 15 Fersolate. Supportive therapy. Pyloric obstruction required surgery	26
36	1954	13 mo.	M	?	2 mo.	Accident. ? × Fersolate. $NaHCO_3$ lavage. Supportive therapy. Pyloric obstruction treated surgically	26

NOTE: No details available on 4 other nonfatal cases.[66] Cases 17 to 21 are probably Fersolate.

* Based on each author's report. No attempt has been made to convert to U.S.P. crystalline Ferrous Sulfate ($FeSO_4 \cdot 7H_2O$). [See text.]

tions of the exsiccated form, the U.S.P. material containing not less than 80% anhydrous salt, $FeSO_4$ exsic. B.P. not less than 77%, and official material in Norway 80.5 to 85%. "Fersolate," for instance, declares 0.2 gm. exsic. $FeSO_4$ (at least 77% anhydrous salt equivalent to about 29% ferrous iron); but a publication from the manufacturer's research laboratories reports the iron content as 24%[71]. Thus, actual dose in terms of anhydrous or U.S.P. crystalline ferrous sulfate or ferrous iron content frequently cannot be determined with any accuracy.

In some instances (Table 5, Nos. 12, 13, 15), the children remained at home with little or no medical care beyond castor oil and reassurance. Lack of appreciation of the reality of ferrous sulfate poisoning by doctors and hospitals makes it necessary to emphasize that ferrous sulfate intoxication may be serious, and that immediate treatment is essential[12,17].

B. *Ferrous Chloride* (*44.06% iron in anhydrous salt, 28.09% in crystalline tetrahydrate*). The use of ferrous chloride in Sweden has resulted in at least 3 cases of iron toxicity. A 2½-year-old girl swallowed about 20 tablets, each containing 0.267 gm. of ferrous chloride (5.34 gm.). The child exhibited typical symptoms of iron poisoning, but

survived. Some residual signs of stomach damage were still evident by roentgenogram 6½ months after ingestion of the tablets and the child's general condition continued poor for a considerable time[45]. In the same report, Lindquist noted a second case of very severe ferrous chloride poisoning following ingestion of about 40 tablets of 0.267 gm. of $FeCl_2$. Extensive necrosis through all the layers of the stomach wall was observed.

More recently a third case, that of a 17-month-old boy, has been reported[56]. The child, while playing with its mother's anti-anemia iron tablets, swallowed an unknown number. No symptoms developed till 4 hours later, and within one-half hour his condition was serious enough to require hospitalization. Methylene blue plus intravenous fluids brought about a decided improvement, but the child's condition once more began to deteriorate so that, in spite of continued therapy, he expired 28 hours after ingestion of the tablets.

Lindquist suggests that the assertion in a pharmacology text that ferrous chloride had no caustic effect and that overdosage involved no risk, was probably based on the paucity of reported cases from ferrous chloride. However, on the basis of the cases reported, he concluded that ferrous chloride, like ferrous sulfate, may prove very dangerous, at least to children.

C. *Ferrous Gluconate* (*12.52% iron in anhydrous salt, 11.58% in dihydrate*). Ferrous gluconate, since the work of Reznikoff and Goebel[60,61] reported in 1937, has become increasingly popular as a source of iron in anemia therapy. It is the most readily absorbed of all ferrous salts[33] and has been found to produce less gastric upset[33,60,61]. Holly[37] recently reported on the administration of ferrous gluconate to pregnant, nonpregnant, anemic and normal females. Patients received as much as 1 gm. per day for up to 76 days without indication of ill effects.

Toxic symptoms are apparently exceedingly rare beyond occasional nausea, and the like, in susceptible individuals, and even here symptoms are less severe than with other forms of iron[37,60]. No reports have been found in the literature of poisoning from this salt, and the medical files of a major producer of this preparation contain no privately reported cases of reactions following overdosages in humans[32]. Further, an English source has indicated recently that 8 ferrous gluconate (Fergon) tablets were ingested, without any untoward effects, by an 11-month-old girl[2]. Clinically, at least, this iron salt appears less irritating and less toxic than other common sources of iron.

D. *Ferric Ammonium Citrate* (*14.5 to 16% iron in the green salt, 16.5 to 18% in the brown form*). Ferric ammonium citrate has become a common medicinal form of iron, since it has been found to be utilized by the body and is lacking in the objectionable astringent properties found in simple ferric salts[5]. However, this salt is not without toxic effects.

In 1949, a 26-year-old pregnant woman took a mixture of 15 gm. of iron ammonium citrate in whiskey, apparently in the hope of inducing an abortion. She died 3 days later of toxic hepatitis[15]. Hurst[41], in 1931, reported a case of iron encephalopathy resulting from iron ammonium citrate. A 58-year-old woman, suffering from anemia, while in the hospital, received 4 × 40 gr. (10 gm.) of the iron salt per day for 23 days. On the 24th day, the daily dose was increased to 2 × 40 gr. plus 2 × 60 gr. (12.5 gm.). Nine times the following morning the patient, while vomiting, lost consciousness. Her breathing became stertorous, the face

was cyanosed, eyes deviated to the right, and pupils dilated. Plantar reflexes were extensor. Between attacks, the patient was semi-conscious and appeared to have a headache. Iron therapy was stopped and the patient gradually recovered.

E. *Ferric Chloride (34.43% iron in anhydrous salt, 20.66% in hexahydrate).* Ferric chloride was the toxic agent employed in 4 homicides reported by Peterson *et al.*[55]. As little as 1½ oz. (45 cc.) of tincture of ferric chloride (6 gm. of salt) proved fatal to an adult male when taken internally. However, in 4 cases involving 1 to 3 oz. of this tincture, the women survived in each case[68]. Ravaglia, in 1884[59], recorded a case of attempted murder with ferric chloride. The woman survived but was troubled by a general dyspeptic disturbance for one month.

Human Lethal Dose. Any attempt to estimate the human fatal dose of these preparations must be made in full recognition of the inherent errors involved. The data are collected from accidental poisoning cases in which it is often difficult or impossible to establish with accuracy the amount consumed. Even though the error may be large, it becomes of interest to examine the summary of the case reports of deaths due to ferrous sulfate as given in Table 5. In several instances reasonably accurate information was available on the amounts of ferrous sulfate ingested. It is possible to make a rough approximation of the fatal dose in terms of mg./kg. of ferrous sulfate for some of the cases.

Death has occurred from the oral ingestion of ferrous sulfate at dosages ranging from 40 to 1600 mg./kg., with an average value of approximately 900 mg./kg. of ferrous sulfate. Comparing this value with those found for the fatal dose of ferrous sulfate in experimental animals it is apparent that this is considerably smaller than the acute oral toxicity value given for the mouse (4500 mg./kg.), guinea pig (1500 mg./kg.) and rabbit (3000 mg./kg.) and of approximately the same magnitude as that given for the cat (>500 mg./kg.) and the dog (800 mg./kg.). It will be noted, of course, that the figure, 900 mg./kg., is based largely on ferrous sulfate poisoning in cases approximately 2 years old and younger. Further consideration of the relative toxicity of ferrous sulfate in man and animals will be taken up again in the presentation of the experimental data from this laboratory[38].

Probable Toxicity Mechanisms. Various mechanisms have been postulated in an effort to explain the cause of death in cases of poisoning following the oral ingestion of iron salts[18,43,58,66,72]. It is difficult to establish that any one factor is solely responsible. Mounting evidence tends to bring into focus the role of the gastrointestinal irritation observed following the fatal ingestion of these preparations. The severe nausea, hematemesis, abdominal cramps and diarrhea followed by the development of profound shock all tend to point to the potentially corrosive effects of these salts as a starting point in the chain of events which leads to a fatal outcome. It has been suggested that the initial effect is a direct corrosion of the gastric mucosa which results in excessive absorption of iron into the systemic circulation with the formation of apoferritin. This then combines with the iron to form ferritin[66], the substance thought to be identical with the vasodepressor material (V.D.M.) found in the blood of animals in experimentally induced shock.

Although vomiting does occur in the human, it does not seem especially reliable as a protective mechanism in iron poisoning. Particularly in young children, the rapidly developing tissue

destruction following the ingestion of large amounts of ferrous salts appears to interfere with these efforts to rid the stomach of massive quantities of iron, often with fatal results. This factor should tend to emphasize the importance of *prompt* and *gentle* gastric lavage combined with vigorous supportive therapy for shock in suspected poison cases. Further, it should stimulate the search for less irritant forms of iron for oral medicinal use.

Summary. The literature on the toxic effects of iron compounds in man and animals is reviewed. The oral median lethal dose in different species has been approximated from published data for the common iron salts. In addition, an estimated fatal dose for humans has been calculated from cases of ferrous sulfate poisoning. Probable mechanisms of toxicity are discussed.

Conclusions. 1. There are adequate data in the literature to establish conclusively that iron salts are toxic to both man and animals. Of 78 cases of poisoning reported in man, 30 ended fatally.

2. The oral toxicity of iron compounds is not a function of the iron content alone, but is dependent upon the particular salt as well.

3. The majority of reported poisonings in man are due to ferrous sulfate. Of the 63 cases reported as due to this salt, 23, or more than one-third, ended fatally. From these data the fatal dose of ferrous sulfate in humans is estimated to be approximately 900 mg./kg.

4. A smaller number of cases of poisoning have been reported after the ingestion of ferrous chloride, ferric chloride and ferric ammonium citrate.

5. No cases of poisoning have been reported from ingestion of ferrous gluconate.

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AN EXPERIMENTAL STUDY OF THE TOXICITY OF FERROUS GLUCONATE

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From an extensive survey of the pharmacological and clinical literature on the toxicity of iron[3] it appeared that ferrous gluconate was less dangerous in overdoses than the other popular iron salts. No cases of poisoning from this iron salt have appeared in the literature, in contrast to the numerous ones with other iron compounds, and particularly with ferrous sulfate. Few pharmacologic data have been published on ferrous gluconate. Therefore, it was decided to carry out a series of studies in our laboratories which would explore and more accurately define this apparent lower experimental and clinical toxicity. Observations on the systemic and local toxicity of ferrous sulfate were included for comparison with the ferrous gluconate results.

Methods. The acute toxicity of ferrous gluconate was determined in direct comparison with that of ferrous sulfate following both intravenous and oral administration in male, albino Swiss mice weighing 22 ± 2 gm. For the intravenous injection, the compounds were administered in aqueous solution in a volume of 0.01 cc./gm. of body weight at a rate of 1.0 cc./minute. A volume of 0.01 cc./gm. of body weight also was used for oral administration. In addition, the acute toxicity of ferrous gluconate was compared with that of ferrous sulfate following oral administration in male, Sprague-Dawley rats weighing 100 ± 10 gms. The compounds in aqueous solution were administered orally in a volume of 1.0 cc./100 gm. of body weight. The mice and rats were observed closely for several hours following injection, and the LD_{50} ± its standard error was estimated at the end of 24 hours by the method of Miller and Tainter[4]. The animals were held under close observation for a period of one week following injection and any delayed manifestations of toxicity were recorded. Where delayed deaths occurred after 24 hours, the LD_{50} was recalculated at the end of the 7-day observation period. Ferrous gluconate and ferrous sulfate were administered orally as a finely divided powder by capsule to cats, weighing 2 to 3 kg., and to mongrel dogs, weighing 7 to 12 kg., in an effort to determine the acute lethal dose following oral administration. After failing to produce fatalities by oral administration of large single doses of either compound, an effort was made to determine whether death occurred following repeated medication with massive oral dosages. Daily doses of 25, 50, 100, 200 and 400 mg./kg. of ferrous sulfate and 100, 200, 400, 800 and 1600 mg./kg. of ferrous gluconate were administered as a powder by capsule to two cats at each dose level 5 days a week for 2 weeks. The cats were observed closely following each medication for evidence of systemic intoxication and the body weights were recorded 3 times a week. All animals were housed in air-conditioned quarters with food and water available at all times, with the exception of the period immediately preceding the oral medications. The mice and rats were fasted for 4 hours and the cats and dogs for 18 hours before oral administration of the ferrous gluconate and ferrous sulfate dosages.

Local tissue toxicity was estimated by means of the trypan blue irritation test procedure[2]. Saline or aqueous-saline solutions of ferrous gluconate from 1% to 8% and ferrous sulfate from 0.25% to 2%, were injected intracutaneously into the abdominal skin of the rabbit followed by the intravenous injection of 10 mg./kg. of trypan blue. The results are expressed in terms of the Threshold Irritant Concentration (TIC) or that concentration, in per cent, which produces no more than a mild irritation (a faint but discernible blue color at the site of injection).

Ferrous gluconate* and ferrous sulfate, U.S.P., were administered as the salt in each case. The results have been calculated in terms of iron in order to provide a more direct comparison of the toxicity values. Percentage factors used for these calculations were as follows:

Ferrous sulfate .7 H_2O = 20.09% iron
Ferrous gluconate .2 H_2O = 11.58% iron

TABLE 1.—ACUTE TOXICITY OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) VERSUS FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN MICE

			$LD_{50} \pm$ s.e. mg./kg.			
			As Salt		As Fe^{++}	
Compound	Route of Adminis.	No. of Animals	24 Hours	7 Days	24 Hours	7 Days
Ferrous sulfate	I.V.	30	65 ± 4.8	51 ± 4.6	13 ± 1	10.2 ± 0.9
Ferrous gluconate	I.V.	40	114 ± 7.6	98 ± 6.8	12.5 ± 0.7	10.8 ± 0.7
Ferrous sulfate	Oral	30	1520 ± 130	1520 ± 130	306 ± 26	306 ± 26
Ferrous gluconate	Oral	60	3700 ± 145	3700 ± 145	429 ± 17	429 ± 17

1. ACUTE TOXICITY STUDIES IN THE MOUSE. a. *Intravenous.* As shown in Table 1, ferrous sulfate was found to be approximately twice as toxic as ferrous gluconate in terms of the salt. When the data were calculated in terms of ferrous iron, there did not appear to be any apparent difference in the acute intravenous toxicity of these two compounds in mice. The value of 13 ± 1 mg./kg. for ferrous sulfate is in almost precise agreement with the value, 13.8 mg./kg. of iron, reported for ferrous sulfate in mice by Edge and Somers[1].

Although a majority of the mice died in the first 24 hours following injection, several deaths occurred in the next 3 to 5 days. No deaths occurred after 5 days. The LD_{50} value for ferrous gluconate at 7 days was not significantly different from the 24-hour value. The 7-day LD_{50} value for ferrous sulfate, however, indicated a significant increase in toxicity due to delayed deaths. In the acute deaths, the mice were severely depressed and lapsed into complete prostration which terminated in a brief clonic convulsive episode with cessation of respiration preceding cardiac arrest. A majority of the acute deaths occurred in one to five minutes after intravenous injection.

b. *Oral.* The acute oral toxicity data in Table 1 show that ferrous gluconate is significantly less toxic than ferrous sulfate, both in terms of the salt and of ferrous iron. The oral LD_{50} for ferrous sulfate was found to be 1520 mg./kg. compared with 3700 mg./kg. of ferrous gluconate which, when expressed in terms of ferrous iron, amounts to 306 mg./kg. as the sulfate and 429 mg./kg. as the gluconate. These differences are statistically significant and indicate that the gluconate, in terms of ferrous iron content, is approximately 40% better tolerated than the sulfate. There were no delayed deaths with either compound following oral administration in mice.

2. ACUTE ORAL TOXICITY STUDIES IN THE RAT. The acute oral toxicity data

*Ferrous gluconate was used in the form of Fergon, supplied by Winthrop-Stearns Inc.

in the rat were found to be of a similar order of magnitude as those found in the mouse as will be noted from the data in Table 2. In terms of the salts, ferrous gluconate was found to be approximately one-third as toxic as ferrous sulfate following oral administration in the rat. When compared in terms of ferrous iron, ferrous gluconate is significantly less toxic, being approximately one-half as toxic as ferrous sulfate. No delayed deaths were observed with ferrous sulfate; one delayed death was observed with ferrous gluconate.

3. ACUTE ORAL TOXICITY IN THE CAT. It was not possible to obtain mortality data by this route of administration at the dose levels employed, since emesis occurred in every cat within 15 minutes to one hour after medication. Severe diarrhea also was observed but became less evident at the higher dosages as the promptness and intensity of emesis increased. It was concluded from these experiments that the acute oral lethal dose of ferrous sulfate in cats was more than 200 mg./kg. and more than 400 mg./kg. for ferrous gluconate.

The pattern of emesis was sufficiently prominent and consistent to permit the estimation of the approximate median emetic dose, AED_{50}, (the approximate dose producing emesis in 50% of the cats) as a criterion for comparing the gastric tolerance to these two compounds in cats. A summary of the emetic effects and of the incidence of diarrhea is given in Table 3. It will be noted that the dose of ferrous gluconate required to produce emesis in 50% of the cats was more than three times as large as that of ferrous sulfate. About twice as much iron in the form of ferrous gluconate was tolerated without vomiting as was tolerated in the form of the sulfate.

4. ACUTE ORAL TOXICITY STUDIES IN THE DOG. Six dogs, one at each dosage level, were given capsules of finely divided ferrous gluconate in amounts ranging from 100 to 3200 mg./kg. Five other

TABLE 2.—ACUTE ORAL TOXICITY OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN RATS

		$LD_{50} \pm$ s.e. mg./kg.			
		As Salt		As Fe^{++}	
Compound	No. of Animals	24 Hours	7 Days	24 Hours	7 Days
Ferrous sulfate	30	1480 ± 184	1480 ± 184	298 ± 37	298 ± 37
Ferrous gluconate	30	4600 ± 560	4500 ± 400	518 ± 63	507 ± 45

TABLE 3.—EFFECTS OF SINGLE ORAL DOSAGES OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN CATS

		Emetic Effects			Diarrhea
		No. Vomited / No. Medicated	AED_{50}, mg./kg.		No. Showing Diar. / No. Medicated
Compound	Dose mg./kg.		As Salt	As Fe^{++}	
Ferrous sulfate	25	1/4	82	16	2/4
	50	2/4	..	..	2/4
	100	1/4	..	..	0/4
	200	4/4	..	..	0/4
Ferrous gluconate	100	0/4	267	31	2/4
	200	1/4	..	..	1/4
	400	4/4	..	..	0/4

dogs received similar capsules of ferrous sulfate in doses from 50 to 800 mg./kg. No deaths or serious evidence of acute systemic intoxication were observed in the dogs at doses up to and including the highest dose level, 800 mg./kg. of ferrous sulfate or 3200 mg./kg. of ferrous gluconate. The most obvious effects produced by these two compounds were emesis and diarrhea (Table 4). Vomiting was noted in the dog receiving 50 mg./kg. of ferrous sulfate but was not encountered in the others until the dose was raised to 800 mg./kg., when a prompt and vigorous emetic reaction was observed. With ferrous gluconate, vomiting did not occur until doses of 1600 and 3200 mg./kg. were reached. A watery diarrhea became apparent approximately one hour after oral administration of 100 mg./kg. of ferrous sulfate and 800 mg./kg. of ferrous gluconate. At doses of 200 and 400 mg./kg. of ferrous gluconate, diarrhea developed the morning of the day following medication.

The occurrence of vomiting and diarrhea, indicative of a protective mechanism similar to that observed in the cat, interfered with the attempt to estimate the acute oral lethal dosage of these compounds in dogs.

5. REPEATED ORAL MEDICATION IN THE CAT. Since it had not been possible to obtain mortality following oral administration of large single doses of either compound in the cat, an effort was made to determine whether death would result from repeated medication with massive hypertherapeutic doses. Daily doses of 25, 50, 100, 200 and 400 mg./kg. of ferrous sulfate and 100, 200, 400, 800 and 1600 mg./kg. of ferrous gluconate were administered as a powder by capsule to 2 cats at each dose level 5 days a week for 2 weeks. No serious body weight changes or mortality occurred among the cats receiving ferrous gluconate. However, one cat on 400 mg./kg. of ferrous sulfate died following the fifth dose. Some impairment of appetite occurred in the second cat at this dose level, but no serious loss in weight occurred and the cat survived the full medication schedule. Occasional vomiting and diarrhea occurred at the lower dosages with both compounds as noted in Table 5. The intensity of the emesis increased with increase of dosage and was associated with a decrease in the incidence of diarrhea. The emesis appeared to be entirely local in effect, since it occurred in less than an hour after medication. Other than the emesis, the cats appeared to suffer no ill effects from the medication. The appetite except at the highest dosages remained normal in every cat.

6. TISSUE IRRITATION STUDIES. Because of the apparent difference in incidence of gastrointestinal irritation observed with these two compounds in cats and dogs, a comparison of their irritant properties was made by means of the trypan blue irritation test[2] with results as summarized in Table 6.

Ferrous gluconate was observed to be distinctly less irritant than ferrous sulfate. The relative irritancy of these two compounds was similar to that observed in the acute oral studies in cats. The TIC (threshold irritation concentration) for ferrous sulfate was found to be 0.25% and for ferrous gluconate four times larger or 1.0%. Recalculation of these data in terms of ferrous iron indicates that the local tissue irritation of ferrous gluconate is less than one-half that of ferrous sulfate. The evidence of a lower local tissue toxicity with ferrous gluconate correlates well with the finding that the acute oral toxicity of ferrous gluconate is significantly less than that of ferrous sulfate upon oral administration to the mouse and rat. In addition, these laboratory results confirm the clinical observations that

dogs received similar capsules of ferrous sulfate in doses from 50 to 800 mg./kg. No deaths or serious evidence of acute systemic intoxication were observed in the dogs at doses up to and including the highest dose level, 800 mg./kg. of ferrous sulfate or 3200 mg./kg. of ferrous gluconate. The most obvious effects produced by these two compounds were emesis and diarrhea (Table 4). Vomiting was noted in the dog receiving 50 mg./kg. of ferrous sulfate but was not encountered in the others until the dose was raised to 800 mg./kg., when a prompt and vigorous emetic reaction was observed. With ferrous gluconate, vomiting did not occur until doses of 1600 and 3200 mg./kg. were reached. A watery diarrhea became apparent approximately one hour after oral administration of 100 mg./kg. of ferrous sulfate and 800 mg./kg. of ferrous gluconate. At doses of 200 and 400 mg./kg. of ferrous gluconate, diarrhea developed the morning of the day following medication.

The occurrence of vomiting and diarrhea, indicative of a protective mechanism similar to that observed in the cat, interfered with the attempt to estimate the acute oral lethal dosage of these compounds in dogs.

5. REPEATED ORAL MEDICATION IN THE CAT. Since it had not been possible to obtain mortality following oral administration of large single doses of either compound in the cat, an effort was made to determine whether death would result from repeated medication with massive hypertherapeutic doses. Daily doses of 25, 50, 100, 200 and 400 mg./kg. of ferrous sulfate and 100, 200, 400, 800 and 1600 mg./kg. of ferrous gluconate were administered as a powder by capsule to 2 cats at each dose level 5 days a week for 2 weeks. No serious body weight changes or mortality occurred among the cats receiving ferrous gluconate. However, one cat on 400 mg./kg. of ferrous sulfate died following the fifth dose. Some impairment of appetite occurred in the second cat at this dose level, but no serious loss in weight occurred and the cat survived the full medication schedule. Occasional vomiting and diarrhea occurred at the lower dosages with both compounds as noted in Table 5. The intensity of the emesis increased with increase of dosage and was associated with a decrease in the incidence of diarrhea. The emesis appeared to be entirely local in effect, since it occurred in less than an hour after medication. Other than the emesis, the cats appeared to suffer no ill effects from the medication. The appetite except at the highest dosages remained normal in every cat.

6. TISSUE IRRITATION STUDIES. Because of the apparent difference in incidence of gastrointestinal irritation observed with these two compounds in cats and dogs, a comparison of their irritant properties was made by means of the trypan blue irritation test[2] with results as summarized in Table 6.

Ferrous gluconate was observed to be distinctly less irritant than ferrous sulfate. The relative irritancy of these two compounds was similar to that observed in the acute oral studies in cats. The TIC (threshold irritation concentration) for ferrous sulfate was found to be 0.25% and for ferrous gluconate four times larger or 1.0%. Recalculation of these data in terms of ferrous iron indicates that the local tissue irritation of ferrous gluconate is less than one-half that of ferrous sulfate. The evidence of a lower local tissue toxicity with ferrous gluconate correlates well with the finding that the acute oral toxicity of ferrous gluconate is significantly less than that of ferrous sulfate upon oral administration to the mouse and rat. In addition, these laboratory results confirm the clinical observations that

ferrous gluconate, being less irritating, is much better tolerated than ferrous sulfate.

Discussion. Comparison of the present acute toxicity data on ferrous gluconate and ferrous sulfate with the data available in the literature indicates some agreement and also some wide discrepancies. The present acute oral LD_{50} values of 1520 ± 130 mg./kg. for ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and 3700 ± 145 mg./kg. for ferrous gluconate ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) in the mouse indicate a higher acute oral toxicity for these substances than that reported for the mouse in the literature.

TABLE 4.—EFFECTS OF SINGLE ORAL DOSAGES OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN DOGS

Compound	*Dose, mg./kg.*		*Vomiting*	*Diarrhea*
	As Salt	*As Fe^{++}*		
Ferrous sulfate	50	10.0	Yes	No
	100	20.1	No	Yes at 2 hours
	200	40.2	No	Yes at 1 hour
	400	80.4	No	Yes at 1 hour
	800	160.8	Yes at 10 min.	Yes at 1½ hours
Ferrous gluconate	100	11.6	No	No
	200	23.2	No	Yes at 24 hours
	400	46.4	No	Yes at 24 hours
	800	92.8	No	Yes at 1½ hours
	1600	185.6	Yes at 1 hour	Yes at 2 hours
	3200	371.2	Yes at 1½ hours	Yes at 1 hour

TABLE 5.—EFFECTS OF REPEATED MASSIVE ORAL DOSAGE (5 DAYS A WEEK FOR 2 WEEKS) OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN CATS

Compound	*Dose, mg./kg.*		*Mortality*	*Emetic Effects*	*Diarrhea*
	As Salt	*As Fe^{++}*			
Ferrous sulfate	25	5.0	0/2	Occasional, one cat	None
	50	10.0	0/2	Occasional, one cat	Occasional, both cats
	100	20.1	0/2	Frequent, both cats	Occasional, both cats
	200	40.2	0/2	Frequent, both cats	Frequent, both cats
	400	80.4	1/2 (7th day)	Daily	None
Ferrous gluconate	100	11.6	0/2	Occasional, one cat	Occasional, one cat
	200	23.2	0/2	Occasional, both cats	Frequent, both cats
	400	46.4	0/2	Frequent, both cats	Occasional, both cats
	800	92.8	0/2	Frequent, both cats	Occasional, both cats
	1600	185.6	0/2	Daily	Occasional, both cats

TABLE 6.—TRYPAN BLUE IRRITATION DATA ON FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) VERSUS FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$)

Compound	*Concentration in Per Cent (as salt)*	*Maximum Av. Irritation Score*	*Adjective Rating*	**TIC, %*	
				Salt	*Fe^{++}*
Ferrous sulfate	0.25	1.3	Mild	0.25	0.05
	0.5	7.3	Moderate		
	1.0	16.0	Marked		
	2.0	16.0	Marked		
Ferrous gluconate	1.0	3.3	Mild	1.0	0.12
	2.0	5.3	Moderate		
	4.0	13.3	Marked		
	8.0	16.0	Marked		

* TIC—Threshold irritant concentration.

These variations may be due to differences in methods of administration, strain of mice, conditions of assay, and the like. The acute intravenous toxicity for ferrous sulfate as ferrous iron, 13 ± 1 mg./kg., however, agrees almost precisely with the reported literature value of 13.8 mg./kg.[1] In the case of the cat, a literature value of greater than 500 mg./kg. of ferrous sulfate was reported. In the present study, no mortality was observed with single oral doses of ferrous sulfate up to and including 200 mg./kg. When given by repeated oral administration, however, one of two cats died at the end of the first week at a dose of 400 mg./kg. of ferrous sulfate. An estimated acute oral lethal dose of 800 mg./kg. of ferrous sulfate for the dog has been reported[5]. In the present study no mortality was observed following oral administration of ferrous sulfate at dosages up to and including 800 mg./kg. in the dog. Copious vomiting was encountered in both the cat and the dog, which tended to interfere with attempts to estimate the acute oral lethal dose of ferrous sulfate in these two species.

The fact that copious and effective emesis interfered with the estimation of the acute oral lethal dose of ferrous sulfate in both the cat and dog indicates that this protective mechanism may be better developed in these two species than it is in the human. It is of interest to note that the estimated oral median lethal dose of 900 mg./kg. for ferrous sulfate in children, referred to earlier[3], is within the limits of experimental error for the acute oral LD_{50} values for ferrous sulfate in the mouse (1520 ± 130 mg./kg.) and the rat (1480 ± 184 mg./kg.) as established in the present investigation.

Summary. The results of a direct comparison of the acute systemic and local toxicity of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and ferrous gluconate ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) in experimental animals may be summarized as follows:

1. Studies in mice indicate that the acute intravenous toxicity of ferrous gluconate (114 ± 7.6 mg./kg.) is approximately half that of ferrous sulfate (65 ± 4.8 mg./kg.) in terms of absolute weights of the salts. In terms of iron, however, there is no apparent difference in the toxicity of the two compounds by this route of administration. Delayed deaths occurred with both compounds but were significantly greater with ferrous sulfate.

2. Lower toxicity was observed with both compounds when given orally to mice. The acute oral toxicity values (ferrous sulfate, 1520 ± 130 mg./kg.; ferrous gluconate, 3700 ± 145 mg./kg.) were more than twenty times as large as those following acute intravenous injection. In the rat ferrous gluconate (4600 ± 560 mg./kg.) was only one-third as toxic as ferrous sulfate (1480 ± 184 mg./kg.) as the salt and one-half as toxic in terms of ferrous iron. No delayed deaths of significance were observed following oral administration in either species.

3. Attempts to estimate the acute oral toxicity in cats were unsuccessful, due to intense local gastric irritation which resulted in prompt and copious vomiting. Approximately twice as much ferrous iron in the form of ferrous gluconate as ferrous sulfate was tolerated before vomiting occurred.

4. In the dog the acute oral median lethal dose was estimated to be greater than 800 mg./kg. of ferrous sulfate and more than 3200 mg./kg. of ferrous gluconate. No deaths or serious evidence of acute systemic intoxication were observed at these doses. The emesis and diarrhea produced by both compounds rendered attempts to estimate accurate LD_{50} values impracticable.

5. Local tissue irritation studies indicated that twice as much iron in the

form of ferrous gluconate could be intracutaneously injected without serious damage as could be tolerated in the form of the sulfate.

6. Daily oral administration of ferrous gluconate powder by capsule to cats, 5 days a week for 2 weeks at the hypertherapeutic dosages of 100 to 1600 mg./kg. produced no mortality and no evidence of cumulative toxicity. Emesis and diarrhea were noted at all dose levels. Emesis was particularly prompt and copious at the highest dose levels.

7. Similar daily oral administration of ferrous sulfate to cats at doses of 25 to 400 mg./kg. resulted in death of one of two cats at the 400 mg. level at the end of the first week. Other than emesis and diarrhea, no additional serious toxic effects were noted. No cumulative toxic effects were observed.

8. The magnitude of the acute oral toxicity values when compared with the acute intravenous figures in mice indicates a relatively low order of absorption from the intestinal tract. An additional safety factor is evident from the oral studies in the cat and the dog in which the local irritant effects induce a protective emesis. These data suggest prompt, gentle gastric lavage along with supportive therapy for shock as an effective emergency measure in those cases where, for any reason, vomiting does not occur spontaneously following oral ingestion of ferrus sulfate, ferrous gluconate or other soluble iron salts.

9. These studies clearly establish that ferrous gluconate is less irritating and less toxic than ferrous sulfate when considered from the standpoint of the total weight of drug administered or in terms of their iron contents. A firm experimental basis for the lack of clinical toxicity and for the therapeutic preference for ferrous gluconate, therefore, appears to be demonstrable.

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SUMMARIO IN INTERLINGUA

Un Studio Experimental del Toxicitate de Gluconato Ferrose

Le sequente es un summario del resultatos de un comparation directe del acute toxicitate systemic e local de sulfato ferrose ($FeSO_4 \cdot 7H_2O$) e gluconato ferrose ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) in animales experimental:

1. Studios in muses indica que le acute toxicitate intravenose de gluconato ferrose (114 ± 7,6 mg/kg) es approximativemente un medietate del acute toxicitate intravenose de sulfato ferrose (65 ± 4,8 mg/kg), super le base del pesos absolute del sales. Super le base del contento de ferro, del altere latere, il ha nulle apparente differentia in le toxicitate del duo compositos in administrationes intravenose. Mortes retardate occurreva con ambe compositos, sed illos esseva significativemente plus numerose con sulfato ferrose.

2. Pro ambe compositos, un plus basse toxicitate esseva observate in muses quando le administration esseva effectuate per le via oral. Le pesos de acute toxicitate oral (sulfato ferrose, 1520 ± 130 mg/kg; gluconato ferrose, 3700 ± 145 mg/kg) esseva plus que 20 vices plus grande que illos del acute toxicitate intravenose. In rattos le toxicitate de gluconato ferrose (4600 ± 560 mg/kg) esseva solmente un tertio del toxicitate de sulfato ferrose (1480 ± 184 mg/kg), super le base del pesos absolute del sales, e un medietate, super le base del ferro ferrose. Mortes retardate non esseva observate in numeros significative post le administration oal in o le muses o le rattos.

3. Nos non succedeva a estimar le acute toxicitate oral del duo compositos in experimentos con cattos proque iste animales reageva per un intense irritation gastric local que resultava in un prompte e copiose vomito. Circa duo vices el quantitate de ferro ferrose esseva tolerate in le forma de gluconato ferrose que in le forma de sulfato ferrose ante que le vomito occurreva.

4. In canes le peso median del acute dose mortal in administrationes oral esseva estimate como supra 800 mg/kg de sulfato ferrose e supra 3200 mg/kg de gluconato ferrose. Con iste doses nulle mortes o serie signos de acute intoxication systemic esseva observate. Le emesis e le diarrhea producite per ambe compositos non permitteva le determination de exacte valores.

5. Studios de histo-irritation local indicava que duo vices le quantitate de ferro in le forma de gluconato ferrose que in le forma de sulfato ferrose poteva esser injicite intracutaneemente sin resultante damnos seriose.

6. Le diurne administration oral de pulvere de gluconato ferrose in capsulas; 5 dies per septimana, durante 2 septimanas, in doses hypertherapeutic de inter 100 e 1600 mg/kg; non produceva ulle mortalitate in cattos. In iste experimentos nulle prova de toxicitate cumulative esseva observate. Emesis e diarrhea esseva notate con omne nivellos de dosage. Al plus alte nivellos, emesis esseva specialmente prompte e copiose.

7. In simile experimentos con cattos, diurne administrationes oral de sulfato ferrose in doses de inter 25 e 400 mg/kg resultava in le morte de un ex duo cattos al fin del prime septimana de administrationes al nivello de 400 mg/kg. Foras del emesis e del diarrhea nulle altere serie effectos esseva notate. Nulle toxicitate cumulative esseva observate.

8. Le magnitude del valores de acute toxicitate oral in muses comparate con le magnitude del valores de acute toxicitate intravenose indica un relativemente basse grado de absorption ab le tubo intestinal. Un factor de securitate additional es evidente ab le studios oral in cattos e canes in que le irritante effectos local induce un emesis protective. Iste datos suggere un prompte e dulce lavage gastric insimul con therapia supportative pro choc como un efficace prime mesura in casos in que pro un o altere ration emesis non occurre spontaneemente post ingestion oral de sulfato ferrose, gluconato ferrose, o alicun altere solubile sal de ferro.

9. Iste studios establi clarmente le facto que gluconato ferrose es minus irritante e minus toxic que sulfato ferrose, considerate tanto ab le puncto de vista del peso total del droga administrate como etiam ab le puncto de vista del contento de ferro. Assi il pare possibile demonstrar un firme base experimental pro le absentia de toxicitate clinic de gluconato ferrose e pro su preferibilitate therapeutic.

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THE PREPARATION OF FERROUS GLUCONATE AND ITS USE IN THE TREATMENT OF HYPOCHROMIC ANEMIA IN RATS

PAUL REZNIKOFF AND WALTHER F. GOEBEL

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The parenteral administration of iron compounds has been limited by their toxic effects on tissues. It has been difficult to procure an iron derivative which, when injected into animals, does not cause local lesions, often of a necrotic nature. When an iron salt is used, in which iron is in the anion, such as potassium ferrocyanide, no such injury occurs. This salt is excreted readily by the kidney, however, and cannot therefore be employed in the study of iron metabolism.

After investigating many iron compounds in search of a derivative which does not cause the precipitation of protein, and at the same time affords the body a source of iron readily convertible into hemoglobin, ferrous gluconate was finally prepared. This substance is stable in the solid form in the presence of light and air, is readily soluble in water and solutions of the salt do not precipitate protein. Freshly prepared solutions of ferrous gluconate are colorless but on shaking or standing in the presence of air, soon become green. This suggests that oxidation of the ferrous iron to the ferric state has occurred. Nevertheless, when this partially oxidized solution is added to blood serum, no precipitation of protein ensues. When such solutions are injected into a rat or a human being no local or general reactions occur and no induration is obtained around the site of the injection. In man the injection of ferrous gluconate solutions causes no more discomfort than does the intramuscular introduction of

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causes neither undue local injury nor
of absorption when iron is given by
for studying iron metabolism.

EXPERIMENT

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the air. Four hundred and fifty g
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$(C_6H_{11}O_7)_2Ba \cdot H_2O$. Calculated Ba,
per cent. $(\alpha)_D = +8.0$ in water

2. Ferrous gluconate. Two hundr
were dissolved in 1 liter of boilir
placed in a 2 liter wide-mouthed E
three-hole rubber stopper, bearing
the bottom of the flask. The thi
served as an opening through whi
the flask could be removed with a p
was now gently bubbled through th
a solution of 102.0 grams (1 mol
grade) ($FeSO_4 \cdot 7H_2O$) dissolved in
added. The reaction mixture was

F FERROUS GLUCONATE
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ANEMIA IN RATS

ND WALTHER F. GOEBEL

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any medicament for therapeutic purposes. Since this substance causes neither undue local injury nor presents the uncertain factor of absorption when iron is given by mouth, it obviously is ideal for studying iron metabolism.

EXPERIMENTAL

The preparation of ferrous gluconate

1. Barium gluconate. Four hundred grams of crystalline calcium gluconate were dissolved in 3 liters of water at 90°C. The calcium was precipitated from solution by the addition of exactly one equivalent of oxalic acid $(COOH)_2 \cdot 2H_2O$ or 117.3 grams of crystalline acid dissolved in 300 cc. of hot water. The precipitated calcium oxalate was removed by centrifugation, and the precipitate washed twice with water. The clear solution and washings, which at this point should contain neither free oxalic acid nor calcium ions, were combined and neutralized to pH 8.5 with a solution of 293.5 grams of barium hydroxide, $Ba(OH)_2 \cdot 8H_2O$ (1 mol) dissolved in 1 liter of hot water. The solution was concentrated *in vacuo* to a volume of approximately 1200 cc. and allowed to stand at 0°C. for 48 hours. The crystalline barium gluconate which separated was filtered and dried in the air. Four hundred and fifty grams of substance were recovered. Barium gluconate crystallizes under these conditions with one molecule of water of crystallization. Analysis: $(C_5H_{11}O_5COO)_2Ba \cdot H_2O$. Calculated Ba, 25.22 per cent. Found 25.15 per cent. $(\alpha)_D = +8.0$ in water (C = 1.5 per cent).

2. Ferrous gluconate. Two hundred grams of barium gluconate were dissolved in 1 liter of boiling water. The solution was placed in a 2 liter wide-mouthed Erlenmeyer flask fitted with a three-hole rubber stopper, bearing two glass tubes leading to the bottom of the flask. The third hole, 12 mm. in diameter, served as an opening through which samples of the content of the flask could be removed with a pipette. Oxygen-free nitrogen was now gently bubbled through the solution. After 15 minutes, a solution of 102.0 grams (1 mol) of ferrous sulfate (reagent grade) $(FeSO_4 \cdot 7H_2O)$ dissolved in 250 cc. of warm water was added. The reaction mixture was so adjusted that neither free

barium nor sulfate ions could be detected. The solution was carefully syphoned into four centrifuge bottles, each containing approximately 30 cc. of toluene. The delivery tube was placed below the toluene layer, in order to minimize oxidation of the sensitive ferrous gluconate. The solution was now centrifuged to remove the precipitated barium sulfate. The clear, pale green solution of ferrous gluconate was carefully syphoned into a Claissen distilling flask containing 100 cc. of toluene, and the solution concentrated in an atmosphere of nitrogen to a volume of 600 cc. The syrupy liquid was quickly transferred to an 800 cc. beaker and placed in a desiccator. The air in the desiccator was replaced with nitrogen and the vessel placed in the ice-chamber. After 48 hours at 0° the crystalline ferrous gluconate was filtered with suction as rapidly as possible from the greenish mother liquors. The crystalline product was repeatedly washed with small portions of cold 50 per cent alcohol, and finally with alcohol and ether. One hundred and fifty-three grams of pure ferrous gluconate were recovered, or 90 per cent of the theoretical yield. Analysis: $(C_5H_{11}O_5COO)_2\ Fe \cdot H_2O$. Calculated Fe 12.05 per cent. Found Fe 12.10 per cent. $(\alpha)_D = +3.5°$ in water (C = 2.5 per cent).

Total iron was determined by digesting a weighed sample of ferrous gluconate (0.2 gram) with 4 cc. of concentrated H_2SO_4 and 5 cc. of 30 per cent H_2O_2. Five cubic centimeters of concentrated HNO_3 were added after digestion to insure complete oxidation of the iron to the ferric state. The iron was precipitated as ferric hydroxide by the addition of concentrated ammonia. After filtering off the ferric hydroxide, the latter was dissolved in dilute HCl and the iron determined iodimetrically in the usual manner. The small quantities of ferric iron present in the ferrous gluconate were determined directly by iodimetric titration in an atmosphere of nitrogen.

Properties of ferrous gluconate

Ferrous gluconate crystallizes from water with one molecule of water of crystallization. In the absence of oxygen the compound separates as pale greenish yellow needles. The crystalline

substance can be kept in the sol
many months without decom
ferrous gluconate have been n
in no instance was more than
in the final product. In severa
iron was present. Analysis
made several months after st
stoppered bottles showed no
gluconate can be recrystallize
from water or from 50 per cen
soluble in water at 25°C.

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Administration of f

As a preliminary to the us
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albino rats. One animal devel
ment and was discarded. Of

treated and kept as controls; 8 received daily injections of ferrous gluconate; and 7 were given the substance daily by mouth. All the rats were born of milk-fed mothers and were fed on an exclusive diet of milk and klim throughout the experiment. At the age of 2 to 3 months most of the rats had developed an anemia sufficiently pronounced so that the possible efficacy of ferrous gluconate could be studied. A solution of the compound was prepared so that 0.2 cc. contained 1 mgm. of iron and this

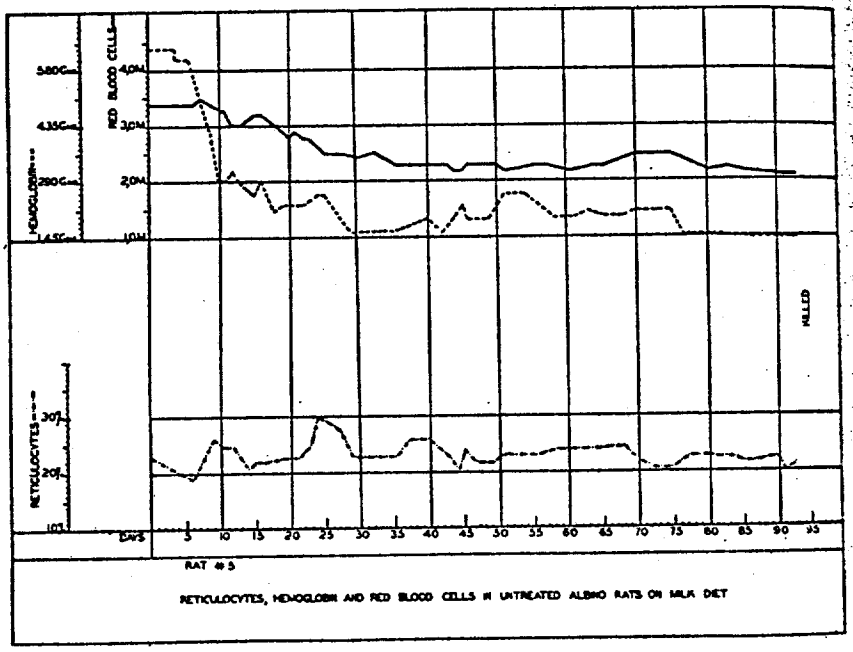

CHART 1

amount was fed daily by a medicine dropper or was injected intramuscularly. In the earlier experiments the ferrous gluconate contained small quantities of ferric iron but later preparations contained no trace of ferric iron. Four of the injected animals and 2 of those fed by mouth received the latter substance.

Charts 1, 2 and 3 illustrate typical results obtained with untreated, fed and injected animals respectively.

As soon as the animals given ferrous gluconate parenterally

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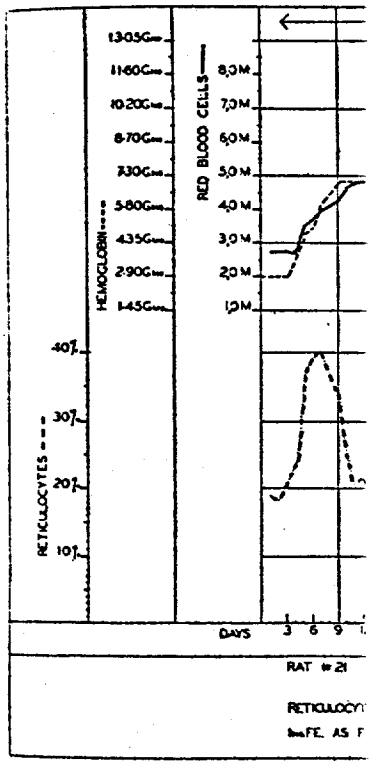

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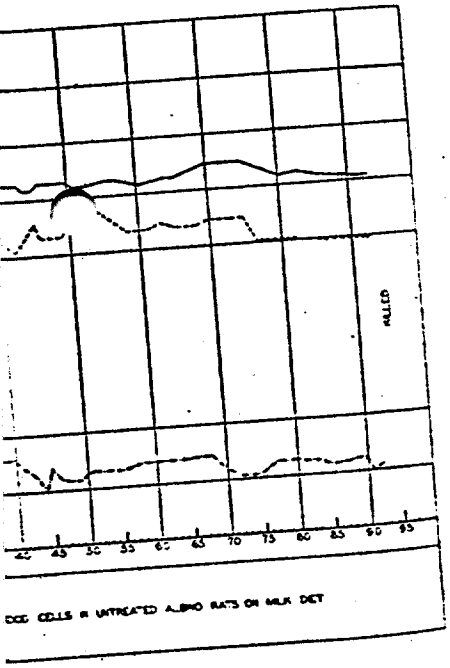

ART 1

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presented normal red blood cell and hemoglobin values, the injections were stopped and the administration of ferrous gluconate was continued by mouth. No increase in reticulocytes was obtained. When the rats which had been fed ferrous gluconate had attained a high hemoglobin value, the iron salt was given parenterally. In two of these a low reticulocyte count had been

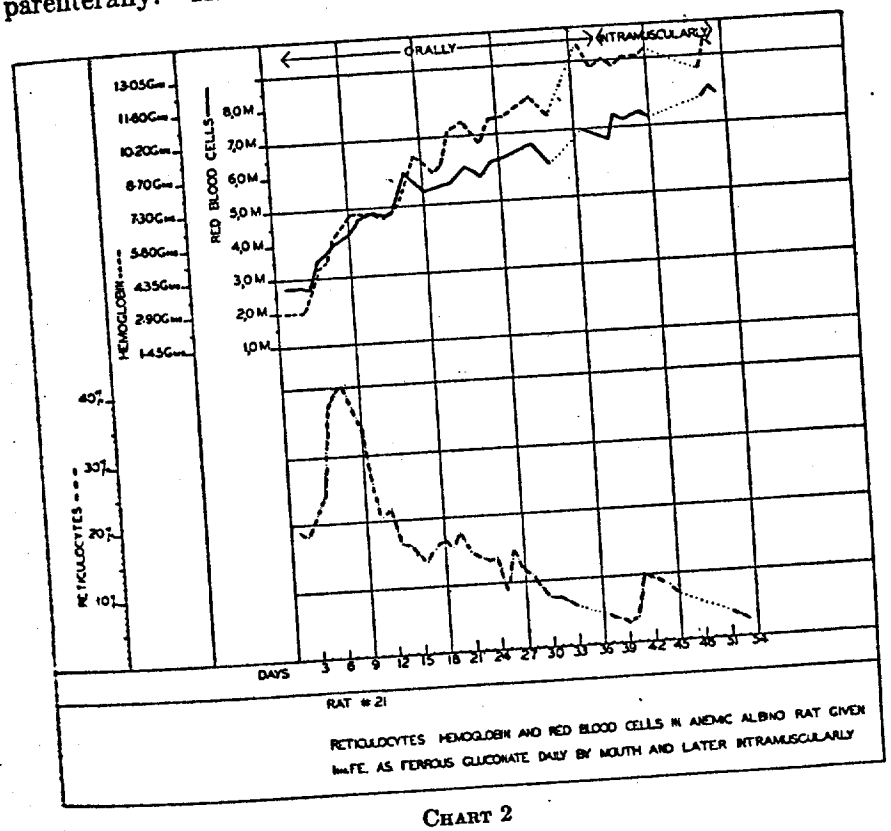

CHART 2

reached before the injections were made. Following the injections further reticulocyte, red blood cell and hemoglobin rises occurred. In the case of 3 other animals injections were started before the reticulocytes had reached a low level; 2 showed a further increase in hemoglobin values, and 1 in red blood cell count.

An analysis of the response of these rats to the administration of ferrous gluconate is summarized in tables 1 and 2. Rats 2, 3, 6 and 8 were treated with ferrous gluconate containing some ferric salt, since at the beginning of these experiments the method of preparing pure ferrous gluconate had not been entirely perfected. Rats 3 and 8 developed abscesses at the sites of some of

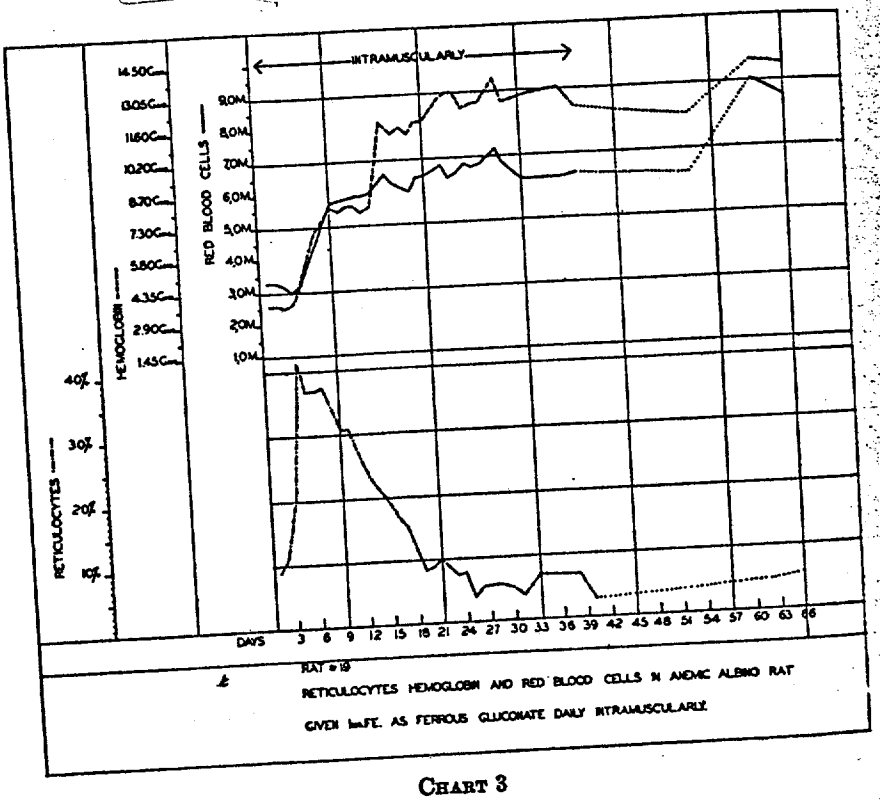

CHART 3

the injections. These animals showed a delay in the rate of hemoglobin formation and rat 3 gave very little hemoglobin per milligram of iron. With these exceptions the tables show that the average increase in hemoglobin per milligram of iron fed is 0.45 gram per 100 cc. of blood. If the extreme values are eliminated and the mean is considered the value is 0.42 gram. In the injected animals the gain in hemoglobin per milligram of iron

TAB

RAT NUMBER	METHOD OF ADMINISTRATION OF FERROUS GLUCONATE	BEGINNING RETICULOCYTE RESPONSE	RETICULOCYTE PEAK	BEGINNING HEMOGLOBIN RISE	MAXIMUM HEMOGLOBIN
		days	days	days	da
4	Orally	2	5	2	2
10	Orally	1	6	2	2
11	Orally	3	5	3	2
12	Orally	4	7	4	2
14	Orally	2	2	2	2
17	Orally	1	5	3	2
21	Orally	1	5	2	2
2	Intramuscularly	3	5	3	1
3	Intramuscularly	2	6	6	1
6	Intramuscularly	3	6	2	
8	Intramuscularly	1	7	4	2
18	Intramuscularly	1	5	4	1
19	Intramuscularly	1	2	3	1
20	Intramuscularly	2	5	2	1
22	Intramuscularly	1	4	2	1

TA

METHOD OF ADMINISTRATION OF FERROUS GLUCONATE	NUMBER OF RATS	BEGINNING RETICULOCYTE RESPONSE		RETICULOCYTE PEAK	
		Minimum	Maximum	Minimum	Maximum
		days	days	days	days
Orally	7	1	4	2	7
Intramuscularly	8	1	3	2	7

* In this summary the results obta not included.

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in tables 1 and 2. Rats 2,
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had not been entirely per-
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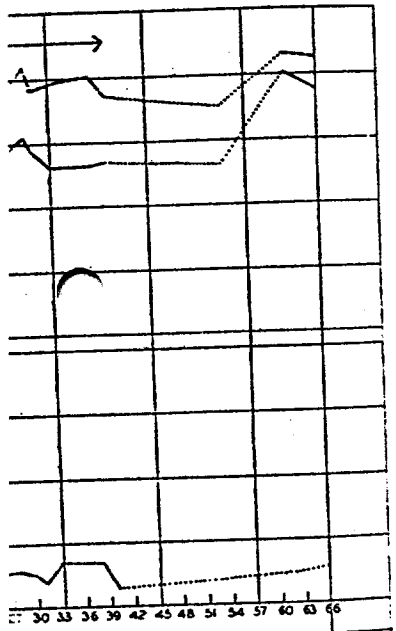

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TABLE 1

RAT NUMBER	METHOD OF ADMINISTRATION OF FERROUS GLUCONATE	BEGINNING RETICULOCYTE RESPONSE	RETICULOCYTE PEAK	BEGINNING HEMOGLOBIN RISE	MAXIMUM HEMOGLOBIN RISE	IRON ADMINISTERED	HEMOGLOBIN GAINED PER 100 CC. OF BLOOD	HEMOGLOBIN UTILIZED PER MILLIGRAM Fe	REMARKS
		days	*days*	*days*	*days*	*mgm.*	*grams*	*grams*	
4	Orally	2	5	2	20	17	7.54	0.44	
10	Orally	1	6	2	22	16	9.28	0.58	
11	Orally	3	5	3	21	18	7.54	0.42	
12	Orally	4	7	4	27	22	9.28	0.42	
14	Orally	2	2	2	21	16	8.12	0.51	
17	Orally	1	5	3	21	18	7.25	0.40	
21	Orally	1	5	2	29	25	8.7	0.35	
2	Intramuscularly	3	5	3	10	9	6.38	0.71	Impure ferrous gluconate
3	Intramuscularly	2	6	6	17	14	4.35	0.31	Impure ferrous gluconate; abscesses
6	Intramuscularly	3	6	2	8	7	5.8	0.83	Impure ferrous gluconate
8	Intramuscularly	1	7	4	21	14	8.41	0.6	Impure ferrous gluconate; abscesses
18	Intramuscularly	1	5	4	14	12	7.25	0.6	
19	Intramuscularly	1	2	3	14	12	8.27	0.69	
20	Intramuscularly	2	5	2	16	14	8.12	0.58	
22	Intramuscularly	1	4	2	14	12	7.25	0.60	

TABLE 2

METHOD OF ADMINISTRATION OF FERROUS GLUCONATE	NUMBER OF RATS	BEGINNING RETICULOCYTE RESPONSE		RETICULOCYTE PEAK		BEGINNING HEMOGLOBIN RISE		MAXIMUM HEMOGLOBIN RISE		HEMOGLOBIN GAINED PER 100 CC. OF BLOOD		HEMOGLOBIN PER MILLIGRAM Fe	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
		days	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*grams*	*grams*	*grams*	*grams*
Orally	7	1	4	2	7	2	4	20	29	7.25	9.28	0.35	0.58
Intramuscularly	8	1	3	2	7	2	6	10	16*	5.8*	8.41	0.58*	0.83

* In this summary the results obtained in the 2 rats which had abscesses are not included.

is 0.62 gram per 100 cc. of blood. Here again, if the extreme values are eliminated, the average mean value is 0.61 gram. Therefore, in these experiments the utilization of injected iron is approximately 50 per cent more effective than is that of iron given by mouth.

DISCUSSION

The actual efficacy of ferrous gluconate in the production of hemoglobin is difficult to determine in these experiments. In the first place no knowledge of the amount of other metals such as copper was available. It is generally recognized that copper has a striking effect upon the acceleration of hemoglobin production from iron. That these rats probably received some copper is suggested by the rather rapid reticulocyte decreases after the peaks were reached (1). In the second place, the total amount of hemoglobin produced, as calculated from a unit volume of blood, depends upon the blood volume and the latter is proportional to the weight of the rats. At the beginning of the experiments the rats, although 2 months old, averaged only 50 grams in weight, because they were maintained on a milk diet. When the experiments were terminated the rats averaged 125 grams in weight. Therefore, the calculated absolute amount of hemoglobin during the experiment is complicated by the change in weight. However, a rough approximation of the utilization of the iron might be made.

The blood volume of a 50 gram rat, by extrapolating the figures given by Donaldson (2), is approximately 3.2 cc. Before iron was given the rats averaged 2.2 grams of hemoglobin per 100 cc. of blood or a total of 0.07 gram. At the end of the experiments the rats' average weight was 125 grams and they had about 7.26 cc. of blood and 10.9 grams of hemoglobin per 100 cc. of blood, or a total of 0.79 gram per rat. In the animals which were fed ferrous gluconate this gain was reached after approximately 22 mgm. of iron were given. In the injected rats 12 mgm. of iron gave an identical increase. The average content of iron in hemoglobin is approximately 0.33 per cent (3, 4, 5, 6). Therefore the percentage of iron converted directly into hemoglobin

in the case of fed rats averag
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If, however, the variables
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Myers (7) obtained a little les
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iron resulted. Two milligram
of hemoglobin per milligram
Nelson (8) gave anemic rats
daily and injected every other
of $Fe(OH)_3$ and observed th
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Sauerwein and Myers (9) obt
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0.42 gram of hemoglobin was
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CON

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in the case of fed rats averaged 11 per cent and, in the injected animals, approximately 20 per cent.

If, however, the variables of weight and blood volume are disregarded, it is interesting to compare the results obtained in these experiments with those of other workers. Beard and Myers (7) obtained a little less than 0.25 gram of hemoglobin per 100 cc. of blood per milligram of iron when $FeCl_3$ was fed at the rate of 1 mgm. daily for 4.9 weeks. When 1.5 mgm. of iron was fed daily for 3 weeks, a little less than 0.32 gram per milligram of iron resulted. Two milligrams of iron fed daily gave 0.40 gram of hemoglobin per milligram of iron in 1.8 weeks. Keil and Nelson (8) gave anemic rats 0.05 mgm. of Cu as $CuSO_4$ orally daily and injected every other day 1 mgm. of iron as a suspension of $Fe(OH)_3$ and observed that in 11 weeks an increase of 0.28 gram of hemoglobin per milligram of iron occurred. Bing, Sauerwein and Myers (9) obtained in 17 days an increase of 0.36 gram of hemoglobin per milligram of iron when 0.5 mgm. was given by mouth daily in the form of $FeCl_3$. In our experiments 0.42 gram of hemoglobin was formed per milligram of iron in 3 weeks in the case of fed rats and 0.61 gram of hemoglobin per milligram of iron in 2 weeks in the injected rats.

CONCLUSIONS

1. The ferrous salt of gluconic acid has been prepared. This compound contains 12 per cent of iron and is soluble in water. Concentrated aqueous solutions of ferrous gluconate do not precipitate protein.

2. When ferrous gluconate is fed to or injected intramuscularly into young anemic albino rats, rapid and marked reticulocyte, red cell and hemoglobin responses are obtained.

3. Ferrous gluconate fed to anemic rats at the rate of 1 mgm. of iron daily caused a hemoglobin response in 3 weeks of approximately 0.4 gram per 100 cc., of blood per milligram of iron. When injected intramuscularly in the same dosage, 0.6 gram of hemoglobin per 100 cc. of blood per milligram of iron was obtained in 2 weeks. These results compare very favorably with those reported in the literature.

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ERRATUM

Hall, V. E., Crismon, J. M., and Chamberlain, P. E.: The Influence of Cold on the Calorigenic Action of Dinitrophenol, 59, 193, 1937.

Page 199, second line below table 2, the word "activating" should be "inactivating."

THE INFLUENCE OF C
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V. E. HALL, J. M. CRIS
From the Department of Phys

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J. Clin. Invest. 16, 1937

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THE USE OF FERROUS GLUCONATE IN THE TREATMENT OF HYPOCHROMIC ANEMIA

By PAUL REZNIKOFF and WALTHER F. GOEBEL

(From the New York Hospital and the Department of Medicine, Cornell University Medical College, and the Hospital of the Rockefeller Institute for Medical Research, New York City)

(Received for publication January 23, 1937)

Although hypochromic anemia due to iron deficiency is universally treated with iron medication, there is not as much unanimity with respect to the particular type of iron compound to be used. The following postulates, however, are accepted: The dosage must be adequate to insure a reasonably rapid increase of hemoglobin, the iron compound, in the dosage given, must be tolerated by the patient without undue distress, and the cost of medication must be within the financial means of the patient.

Many individuals receiving iron complain of such symptoms as nausea, epigastric discomfort, diarrhea or constipation. Some physicians hesitate to subject their patients to possible upset in cases of gastric or duodenal ulcer, colitis, and diarrhea or constipation. It was thought desirable, therefore, to prepare an iron compound which might have minimum irritating effects. One of the characteristics of ferric compounds is their ability to precipitate proteins. Most ferrous compounds are oxidized readily to the ferric state. This precipitating effect on proteins might explain the irritating action of iron compounds on the gastro-intestinal tract of some patients. Ferrous gluconate, prepared anaerobically, was found to have no precipitating action on proteins even when converted into the ferric state. It was decided, therefore, to treat patients with this compound to determine not only its efficacy but also its toxicity compared to medications now in common use. As a further test of its lack of toxic effects, solutions of ferrous gluconate were administered intramuscularly in quantities containing as much as 50 mgm. of iron, with no systemic disturbances and rarely with local discomfort. The method of preparing this substance and its use in treating hypochromic anemia in rats have been described previously (1).

Recently we have been able to simplify the method based upon a report of Neiger and Neuschul (2). These investigators, studying the photochemical reactions of ferrous gluconate, prepared dilute solutions of this salt by boiling aqueous gluconic acid with iron filings. Although these workers do not describe the isolation of the crystalline salt, it occurred to us that crystalline ferrous gluconate might be prepared in quantity and in a high state of purity by employing this simple reaction. Consequently 100 grams of crystalline calcium gluconate[1] were dissolved in 700 cc. of boiling water. A solution of 29.3 grams of oxalic acid dissolved in 150 cc. of warm water was added. The precipitated calcium oxalate was separated by filtration, and the clear filtrate containing gluconic acid was concentrated to 350 cc. in vacuo. The solution of gluconic acid was placed in a one liter, three necked, round bottomed flask bearing a mercury sealed stirrer. One outlet of the flask was fitted with a small water trap to permit the escape of evolved hydrogen gas whereas the third outlet was closed with a rubber stopper. The flask was now heated in a water bath, and the contents rapidly stirred. Twenty-six grams (2 equivalents) of pure powdered iron (Merck's "Iron by Hydrogen") were added. A rapid evolution of hydrogen took place. At the end of two hours the solution in the flask was neutral to litmus paper. The hot solution of ferrous gluconate, colored a pale green, was carefully filtered through a sintered glass funnel of fine porosity. The filtration was conducted in such a manner that at no time did the solution of ferrous gluconate come in contact with atmospheric oxygen. This was accomplished by conducting the solution from the reaction flask by suction into an enclosed filtering system in which all air had been displaced by carbon dioxide.

The solution was allowed to cool in an atmosphere of carbon dioxide and after crystallization of ferrous gluconate was complete, the product was filtered rapidly in a Buchner funnel, washed with a small amount of 50 per cent alcohol, and finally with pure acetone. The substance was placed in a vacuum desiccator to remove all traces of acetone. Eighty-eight grams of ferrous gluconate were recovered.

The product thus obtained is a fluffy white powder with a very slight greenish tint. The substance crystallizes with one molecule of water and contains no detectable ferric iron. The fer-

[1] We have been able to simplify the preparation of ferrous gluconate still further recently by making it from technical gluconic acid.

TABLE I

Summary of results with ferrous gluconate therapy in 13 patients

Case number	Diagnosis	Gastric HCl	Total quantity of iron given	Reticulocyte peak		Initial hemoglobin		Gain in hemoglobin under treatment		Time elapsed before increase in hemoglobin attained	Hemoglobin gain per day		Percentage utilization of iron	Remarks
				Per cent	Days after start of therapy									
			grams			*grams*	*per cent*	*grams*	*per cent*	*days*	*grams*	*per cent*		
1. F. S.....	Hypochromic anemia; post-thyroidectomy; uterine fibroid	Hypochlorhydria	0.400* 2.160†	9.6	7	4.65 8.15	32 56	3.95 3.8	27 26	20 20	0.196 0.189	1.35 1.30	163.0 29.0	B. M. R. −3; menorrhagia continued; referred for hysterectomy
2. G. F.....	Hypochromic anemia; rheumatic heart disease	HCl present	0.225* 2.376†	10.2 9.4	5 4	8.60 10.20	59 70	1.9 2.5	13 17	7 18	0.270 0.138	1.86 0.95	139.0 17.4	Six months previously lost much blood from miscarriage
3. M. N....	Slight hypochromic anemia; rheumatic heart disease		0.156*	3.8	5	10.20	70	1.3	9	12	0.109	0.75	159.0	Convalescing from lobar pneumonia
4. E. S.....	Hypochromic anemia; rheumatic heart disease; uterine fibroid	Achlorhydria	0.475*	6.0	6	7.0	48	3.8	26	21	0.180	1.24	132.0	Hysterectomy after blood was normal
5. D. M....	Ulcerative colitis; ileostomy	Hypochlorhydria	0.400* 3.888† 4.288	22.0	21	7.5	52	6.7	46	55	0.122	0.84	25.8	High reticulocyte count at start of treatment; blood in stools; relapse when therapy stopped
6. M. G....	Duodenal ulcer; hematemesis		1.620†	15.2	2	9.8	67	4.1	28	16	0.251	1.75	41.0	Patient continued to show blood in stools constantly

TABLE I—*Continued*

Case number	Diagnosis	Gastric HCl	Total quantity of iron given	Reticulocyte peak		Initial hemoglobin		Gain in hemoglobin under treatment		Time elapsed before increase in hemoglobin attained	Hemoglobin gain per day		Percentage utilization of iron	Remarks
				Per cent	Days after start of therapy									
			grams			*grams*	*per cent*	*grams*	*per cent*	*days*	*grams*	*per cent*		
7. V. M....	Hypochromic anemia	Achlorhydria	3.780†	9.0	9	5.7	39	8.6	59	38	0.225	1.55	37.5	Profuse menstruation during treatment. Menorrhagia persists after normal count and no medication
8. M. C....	Hypochromic anemia	Hypochlorhydria	2.484†	6.6	4	8.0	55	5.7	39	23	0.250	1.70	37.9	Chief complaint headache which disappeared with normal count
9. M. M....	Hypochromic anemia; uterine fibroid	Achlorhydria	4.212†	7.6	4	8.0	55	5.7	39	39	0.145	1.00	22.3	No drop in count after medication stopped in spite of menorrhagia. During experiment had profuse menstrual flow and upper respiratory infection. Hysterectomy
10. A. M....	Plummer-Vinson syndrome	Hypochlorhydria	3.744†	6.0	9	8.6	59	3.2	22	40	0.080	0.55	14.1	Has developed a normocytic hyperchromic anemia 4 months after normal count reached after faulty nutrition
11. L. V....	Hypochromic anemia	Achlorhydria	2.592†	11.0	8	8.0	55	5.4	37	31	0.173	1.19	34.4	Failed to return to clinic for two three-week periods during study
12. C. DeV..	Hypochromic anemia	Achlorhydria	3.456†	4.6	6	7.9	54	4.5	31	33	0.136	0.94	21.5	Thyroidectomy 3 years previously. B. M. R. +10 at present admission. Reticulocyte count 3.8 per cent at start of therapy
13. R. L. S..	Hypochromic anemia		3.672†	7.4	5	8.3	57	3.8	26	17	0.222	1.53	17.1	Intestinal adhesions

* Intramuscular administration of ferrous gluconate.
† Oral administration of ferrous gluconate.

rous gluconate prepared in this manner is in all respects identical with that previously described. The above method, however, is considerably simpler than that originally described by us for the preparation of crystalline ferrous gluconate (1), and has the additional advantage of being more economical and more easily carried out.

Thirteen female patients ranging in age from 24 to 49 years and averaging 40, and suffering from hypochromic anemia were treated with ferrous gluconate, two by intramuscular injection, eight by oral administration and three by both methods (Table I). In addition, two patients who had demonstrated marked intolerance to other iron compounds were given ferrous gluconate. In most instances, daily reticulocyte, red blood cell and hemoglobin determinations were made until normal values were obtained, and subsequently the blood was studied as frequently as seemed indicated. In this study 14.5 grams of hemoglobin per 100 cc. was equivalent to 100 per cent.

The diagnosis in four of the patients was "idiopathic" hypochromic anemia without complicating factors. In the others, the following conditions were found, in some cases more than one being present: intestinal adhesions, 1; post-thyroidectomy, 2; uterine fibroids, 3; previous miscarriages, 1; rheumatic heart disease, 3; ulcerative colitis and ileostomy, 1; duodenal ulcer, 1.

The initial hemoglobin was less than 7.25 grams per 100 cc., or 50 per cent, in 3 of the patients. In the remaining, the hemoglobin before medication was greater than 7.25 grams. This observation is important since Heath (3) states that a 1 per cent rise in hemoglobin per day is the low limit of a satisfactory response to treatment when the initial hemoglobin is below 50 per cent.

The volume index of one patient who had been bleeding from a duodenal ulcer was 0.97. Another had a volume index of 0.8; in two the determination was not made; and in the other nine, the values varied from 0.58 to 0.76.

Gastric analysis was not performed in 3 cases. Of the rest, 1 had normal hydrochloric acid content after alcohol and histamine; 4 had hypochlorhydria; and 5, achlorhydria.

The anemia in all of these patients responded well to therapy. In only two did the hemoglobin level fail to reach 11.6 grams or 80 per cent and in these the toxicity of ferrous gluconate when given intramuscularly was tested and no attempt was made to complete the treatment with this iron salt. Six of the 13 patients attained hemoglobin values ranging from 13.1 grams (90 per cent) to 14.2 grams (98 per cent); and 5, values ranging from 11.7 grams (81 per cent) to 12.6 (87 per cent).

Of the 13 patients, one had an initial erythrocyte count between 2,000,000 and 2,500,000; one, between 2,500,000 and 3,000,000; four, between 3,000,000 and 3,500,000; one, between 3,500,000 and 4,000,000; two, between 4,000,000 and 4,500,000; and four, between 4,500,000 and 5,000,000. After treatment, three patients had erythrocyte counts between 4,000,000 and 4,500,000; five, between 4,500,000 and 5,000,000; and five, above 5,000,000. Since this response of the red blood cells to treatment showed no abnormalities, this phase of the subject will not be considered further in this report.

Symptomatically, 7 patients were apparently cured with the attainment of a normal blood count. In 4, profuse menstrual bleeding persisted even after a normal blood count was reached. In 2 of these cases hysterectomy was performed (Cases 4 and 9); in the other two Cases 1 and 7), the blood count has been normal for five months in spite of the fact that they have continued to menstruate profusely and have received no medication. One patient (Case 5) who had an ileostomy for ulcerative colitis, became anemic again two months after the administration of ferrous gluconate was stopped although she received large doses of ferrous sulphate and intramuscular liver extract. Another patient (Case 10) returned three months after the cessation of ferrous gluconate therapy with evidences of hyperchromic anemia and a history of severe malnutrition. She is responding well to liver therapy.

The effect of ferrous gluconate therapy is summarized in Table I. An analysis of these results, without a critical consideration of each case, shows that with respect to the reticulocyte count, gain in hemoglobin per day, and percentage utilization of iron, the ferrous gluconate was strikingly effective in the 13 patients treated. Since in this study small oral doses of iron (108 mgm. daily) were

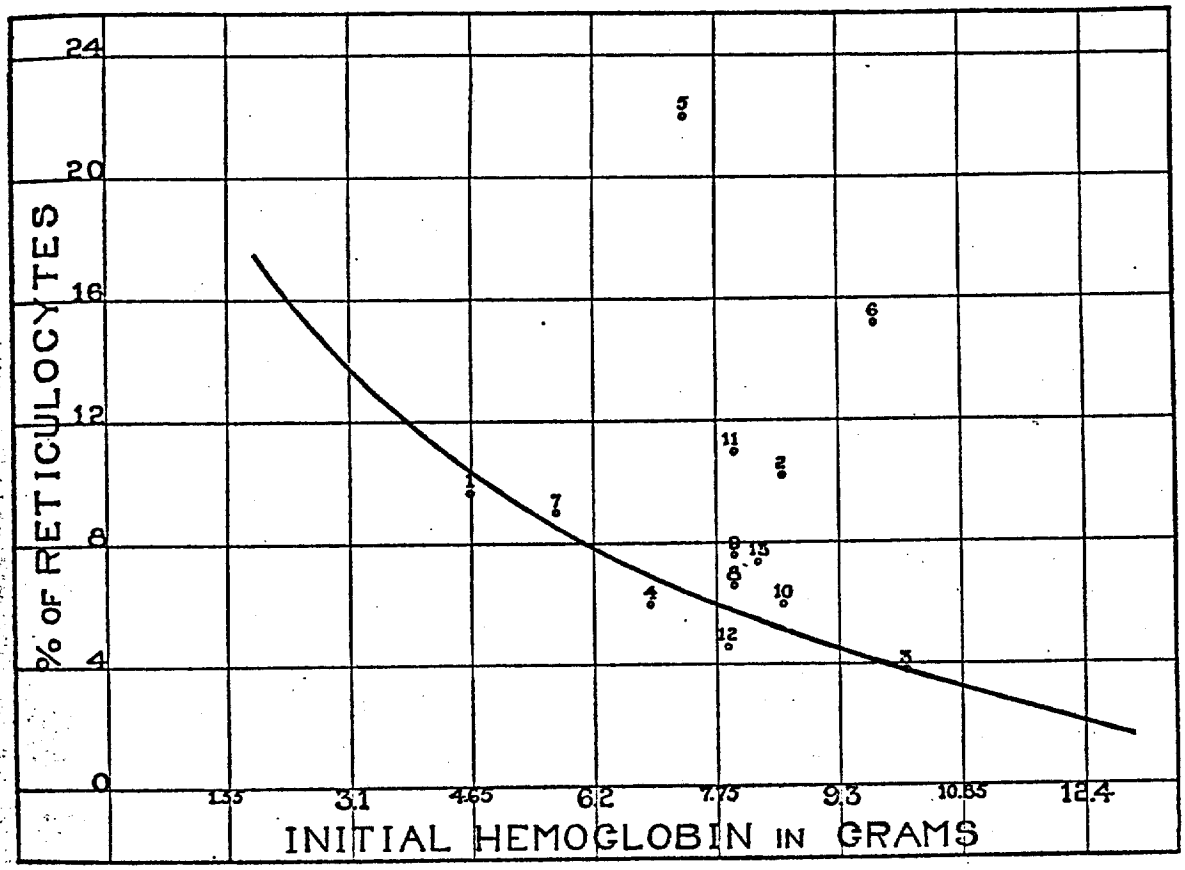

FIG. 1. RETICULOCYTE PEAKS ATTAINED BY PATIENTS PLOTTED ON HEATH'S (3) CURVE OF ADEQUATE RESPONSE

usually administered to determine the response to minimum dosage, the time elapsing before a normal blood count was obtained was not remarkable. However, when for purposes of expediency larger oral doses (216 mgm. daily) were given to Patients 2 and 13, normal blood counts were attained within three weeks. Patients 1, 6 and 8 showed a normal count with only 108 mgm. of iron daily in 20, 16 and 23 days respectively. Case 6, however, represents a posthemorrhagic patient, and the marked response may not be due chiefly to the therapy.

Figure 1 shows the reticulocyte peaks attained by the patients in this study. These are represented by a dot for each case when compared to Heath's curve (3) for adequate reticulocyte response. Patient 5 suffering from ulcerative colitis attained 22 per cent reticulocytes, but her initial count was 10 per cent. Patient 6 who suffered from hematemesis reached a peak of 15.2 per cent from an initial count of 12.6 per cent. Obviously, these results cannot be considered to be entirely due to the treatment. Patient 12 attained a reticulocyte peak of only 4.6 per cent with an initial hemoglobin of 7.9 grams. But preliminary counts were as high as 2.4 per cent, and she had a red blood cell count of 4,800,000 before therapy was started which would tend to lower the peak.

The average daily increase of hemoglobin in the patients receiving ferrous gluconate intramuscularly, exclusive of Patient 5 in whom the results of intramuscular and oral administration overlapped, was 0.189 gram of 1.30 per cent. If Patient 3 is excluded because of her high initial count of 10.2 grams, the results with the remaining three patients, two of whom had initial hemoglobin values of less than 7.25 grams, give an average daily increase of hemoglobin for intramuscular therapy of 0.215 gram or 1.48 per cent. The average daily gain for all patients given ferrous gluconate orally was 0.181 gram or 1.25 per

cent. It is obviously unfair to include Patient 6 in this series as the rapid rise in her hemoglobin following the acute hemorrhage is certainly not due to medication entirely. Patient 2 whose initial hemoglobin before oral therapy was started was 10.2 grams and Patient 10 who subsequently demonstrated a maturative deficiency probably should not be considered in this calculation. If the last two patients are included, the average daily gain of hemoglobin for the group is 0.173 gram or 1.19 per cent; if these two cases are excluded, the average daily gain of hemoglobin is 0.191 gram or 1.32 per cent. Patient 7, the only one in the series whose initial hemoglobin was less than 7.25 grams before oral therapy was started, had a daily hemoglobin gain of 0.225 gram or 1.55 per cent.[2]

The percentage utilization of iron was calculated by multiplying the total gain in grams of hemoglobin per cubic centimeter for each patient by 5000, the approximate adult blood volume, and by 0.0033, the approximate percentage of iron in hemoglobin, and the result obtained was divided by the total amount of iron given the patient. The percentage utilization in the 4 patients who received intramuscular ferrous gluconate calculated in this manner was well over 100 per cent, actually averaging 148 per cent. This is in keeping with the findings of Heath, Strauss and Castle (4) and of Whipple and Robscheit-Robbins (5). Whether this is due to the erroneous assumption of blood volume as the former authors suggest or to a salt effect on iron stored in the body as the latter workers state cannot be determined by our experiments. The percentage utilization of iron in the patients receiving ferrous gluconate by mouth averaged 27.2. If the three patients excluded from the final calculations of the daily hemoglobin increase are likewise omitted from this determination the utilization for oral administration of ferrous gluconate in doses ranging from 0.108 to 0.216 mgm. of iron daily is 28.5 per cent.

The problem of toxicity remains to be considered. It is difficult to obtain objective evidence of intolerance to iron therapy. The following case, for example, which Dr. William Murphy of Boston kindly permits us to cite, illustrates an experience which is occasionally encountered. The patient had suffered from gastric and intestinal disturbances characterized by nausea, constipation and resulting hemorrhoids following the administration of various forms of iron. She also suffered from rectal irritation associated with nocturia, frequency and burning on urination. After taking 0.3 gram of ferrous gluconate three times a day, these symptoms were all produced but to a less degree than with other forms of iron which she tried. Dr. Murphy writes, "I am sure that she could take short courses with this form of iron with less difficulty than any of those which I have previously tried,—and I think it would be well for her to have these capsules for periodic use if it is possible to obtain them."

While this case is not very striking, a second patient offered a better means of studying the relative toxicity of ferrous gluconate. The patient in question was recovering from a Caesarian delivery and her obstetrician hesitated to give her iron for a slight anemia because she had suffered from gastro-intestinal distress and urticaria when she had received iron previously. At one time an injection of some iron compound had produced intense urticaria. When seen 17 days after her operation she had a red blood cell count of 3,600,000 and a hemoglobin of 11.7 grams or 80 per cent. She was not treated at the time but one and one-half months after discharge from the hospital she was given ferrous gluconate in increasing doses until she received 0.9 gram daily containing 108 mgm. of iron with no ill effects. She was then given 35 mgm. of iron in the form of ferrous sulphate and within a few hours suffered a violent gastro-intestinal upset. To determine the effect of the intramuscular injection of ferrous gluconate, she was given 0.45 cc. of a solution containing 25 mgm. of iron per cc. in her left gluteal muscle. For a few hours she had some swelling and soreness but no general reaction and the next morning the local region was practically normal.

At least two patients in this series who suffered no ill effects when taking ferrous gluconate in quantities containing 216 mgm. of iron per day,

[2] After these cases were compiled, a patient was studied who had an initial hemoglobin of 5.7 grams or 39 per cent and was given daily doses of ferrous gluconate containing 324 mgm. Fe. In 11 days her hemoglobin rose to 11.6 grams or 80 per cent, a daily gain of 0.54 gram or 3.7 per cent.

complained of indigestion characterized by anorexia, gas and epigastric distress or of constipation when receiving ferrous sulphate in quantities containing from 180 to 249 mgm. of iron per day.

DISCUSSION

To evaluate the efficacy of ferrous gluconate, a comparison with results obtained with various iron compounds by other workers is necessary. However, it must be remembered that the experimental conditions may not be the same in any two studies. For example, Reimann and Fritsch (6) using ferrous chloride in doses which contained 100 mgm. of iron a day demonstrated remarkable utilization of the iron, ranging from 17 to 45 per cent. However, all their patients had other forms of iron until a few days before the ferrous chloride was started and some demonstrated excellent reticulocyte responses with these other iron compounds which were supposed to give no appreciable hemoglobin increase. It is obvious that the action of the alleged inactive iron salts cannot be disregarded in the final computations. In fact, these authors concluded that all ferrous compounds have approximately the same effect. It is also contrary to the experience of all other workers that large doses of ferric salts are ineffective in hypochromic anemia as Reimann and Fritsch assert. Schulten (7) found that ferrous chloride had to be given in much larger doses and saw no distinct advantage in this iron compound. Davidson (8) reported excellent results with ferrous chloride in doses containing 122 mgm. of iron a day but only 2 of his 7 patients attained 80 per cent hemoglobin. Witts (9) gives as the minimum effective daily dose of ferrous carbonate, an amount containing 300 mgm. of iron. Probably the best comparison of the efficacy of various iron compounds has been made by Fullerton (10). Table II represents a summary of his results compared with those obtained by oral administration of ferrous gluconate. Only those cases are included in which it seems reasonably certain that there are no factors which either interfere with or accentuate the iron effect. This control necessarily makes the available cases few. It is also important to compare separately the results in patients whose initial hemoglobin values were below and above 50 per cent. In our series, patients whose initial hemoglobin readings were below 7.25 grams are rare; in Fullerton's study initial hemoglobin values below 50 per cent were usual. In comparing the effect of the various iron salts it is important to note that all the patients receiving ferrous gluconate orally attained hemoglobin values greater than 11.6 grams or 80 per cent, while in Fullerton's series 9 of the 15 patients treated with ferrous sulphate and 2 of the 3 taking ferrous carbonate for whom data is given failed to reach such a level.

TABLE II

Relative efficacy of various iron compounds *

Compound used	Daily iron dosage	Initial hemoglobin	Number of cases	Average daily hemoglobin rise	Average time before hemoglobin rise	Utilization of iron
	grams	*per cent*		*per cent*	*days*	*per cent*
Ferrous sulfate....	0.180	<50	12	1.175	30	15.70
Ferrous sulfate....	0.120	>50	3	0.650	34	13.00
Iron ammonium citrate.....	1.215	<50	30	1.270		2.50
Iron ammonium citrate.....	1.215	>50	3	1.030		2.03
Ferrous carbonate	0.110	<50	6	0.955		20.80
Ferrous carbonate	0.110	>50	3	0.520		11.30
Ferrous carbonate	0.220	<50	8	0.863		8.80
Ferrous carbonate	0.220	>50	1	0.180		1.96
Ferrous carbonate	0.330	<50	10	1.125		8.18
Ferrous carbonate	0.330	>50	3	0.940		6.84
Ferrous chloride..	0.132–0.198	<50	4	1.420		20.70
Ferrous chloride..	0.132–0.198	>50	3	1.000		14.50
Ferrous gluconate	0.108	<50	1	1.550	38	37.50
Ferrous gluconate	0.108	>50	5	1.230	29	29.00
Ferrous gluconate	0.216	>50	2	1.240	18	17.25

* 14.5 grams = 100 per cent hemoglobin.

Since Barkan's (11) and Meulengracht's (12) reports, most clinicians feel that large doses of iron are essential in treating hypochromic anemia (13, 14, 15, 3, 16). Whipple and Robscheit-Robbins (17), working with standard anemic dogs, emphasize the fact that the particular type of iron is unimportant as long as it is given in large doses. However, Furth and Scholl (18) found that ferrous salts are much more easily absorbed from intestinal loops of rabbits than ferric compounds and most workers who advocate large doses of iron admit that ferrous salts are more efficiently utilized in patients (14, 3, 16). Isaacs, Goldhamer, Isaacs and Sturgis (19) feel that very little is known about soluble iron salts. The results of most studies show, however, that large doses of iron may not produce a normal blood count for a considerable time (15) and occasionally rather severe

doses of iron will cause surprising improvement (20).

The real problem of iron therapy is not the theoretical utilization of iron, or the reticulocyte response, or even the daily increase of hemoglobin for any particular period of treatment. These are important only as they indicate the return of the patients' blood to normal in a reasonably short time without undue inconvenience. Most patients suffering from hypochromic anemia respond well to most forms of iron when administered in adequate dosage. For the patients who cannot tolerate the usual iron compounds, it is important to have a medication which is effective and which causes minimum disturbance. For all patients in need of iron it is desirable to use a compound which gives good results with the least discomfort. Ferrous gluconate seems to be such a medicament.

CONCLUSIONS

1. Ferrous gluconate prepared in the absence of oxygen has been used in the treatment of 13 patients suffering from hypochromic anemia.

2. The use of ferrous gluconate compared with other iron preparations results in satisfactory reticulocyte responses, a high percentage utilization of iron, and such daily increase in hemoglobin that a normal level occurs in a reasonably short time.

3. Four patients, who showed toxic reactions to other iron compounds, were able to take ferrous gluconate without any undue distress.

4. In the patients who received ferrous gluconate intramuscularly up to the present no systemic and only rare and mild local reactions occurred. However, in view of the efficacy of the oral administration of ferrous gluconate and its lack of toxicity there is seldom any reason for its parenteral administration.

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Am. J. Dis. Child., 1955

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TETANY SYNDROME IN NEWBORN INFANTS

REMOTE DEPOSIT OF CALCIUM SALTS FOLLOWING INJECTION OF CALCIUM GLUCONATE

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The local precipitation of calcium salts following intramuscular injection of calcium gluconate during treatment of tetany in newborn infants has been reported several times. So far I have seen no paper which mentions the occurrence of abnormal deposits within the body at a place remote from the original site of injection. Because of the rather frightening possibilities that such an eventuality affords, it is my purpose here to report 2 cases in which this phenomenon was observed.

The 2 papers that have come to my attention to date which record in detail the local deposit of calcium salts after injection of calcium gluconate are those of von Hofe and Jennings [1] and Tumpeer and Denenholz.[2] The former authors reported that calcium gluconate was introduced into both thighs and into the left infrascapular region. Calcium precipitation occurred in all three areas, and in the thighs sloughs developed, from which a green streptococcus was recovered. Gradual resorption was complete by the age of 2 months and 3 weeks. The latter authors also reported that sloughs developed, from which a chalky mineral substance was extruded. This was identified as calcium phosphate. Both calcium gluconate and calcium levulinate had been administered. Recently Bakwin [3] mentioned having seen this phenomenon occur, and I have observed it at least a half-dozen times. The occurrence is therefore probably far from rare.

REPORT OF CASES

CASE 1.—The patient, a boy, was born by low forceps delivery after difficult labor. During the course of treatment of severe tetany with associated general and cerebral edema, 10 cc. of a 10 per cent solution of calcium gluconate was injected into the muscles of the right thigh. The early soft swelling that this induced

1. von Hofe, F. H., and Jennings, R. E.: Calcium Deposition Following the Intramuscular Administration of Calcium Gluconate, J. Pediat. **8**:348 (March) 1936.

2. Tumpeer, I. H., and Denenholz, E. J.: Calcium Deposit Following Therapeutic Injections in Tetany of the New Born, Arch. Pediat. **53**:215 (April) 1936.

3. Bakwin, H.: Pathogenesis of Tetany of the New Born, Am. J. Dis. Child. **54**:1211 (Dec.) 1937.

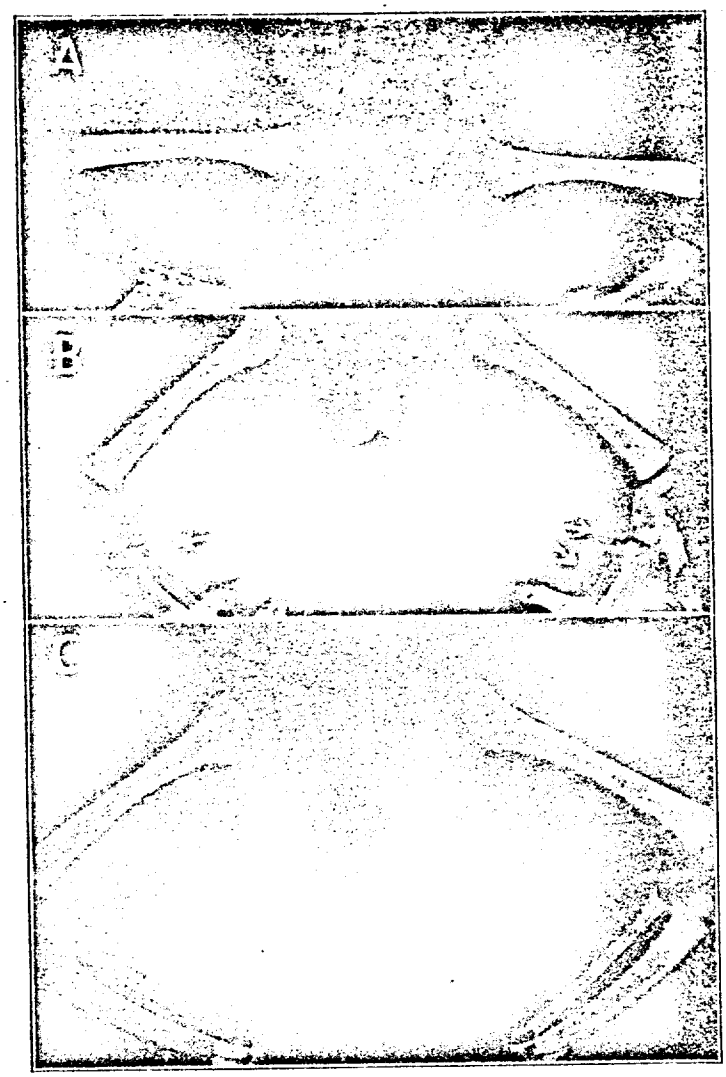

Fig. 1 (case 1).—*A*, roentgenogram showing the deposits of calcium throughout the muscles of the right thigh on the twenty-seventh day of life, nineteen days after the intramuscular injection of 10 cc. of a 10 per cent solution of calcium gluconate. *B*, roentgenogram taken thirty-five days after *A*, showing almost complete absorption of the calcium from the right thigh. The arrows point to blood vessels about both knee joints, visible presumably because of the deposits of calcium salts within their coats. Scattered deposits of calcium along the shaft of the left femur may or may not be within the blood vessels of this region. *C*, roentgenogram taken two months and five days after *B*. Precipitated calcium is no longer demonstrable.

was gradually replaced by an almost stony hardness, which persisted long after the tetany syndrome was under control. A total of 5.25 cc. of parathyroid extract had been injected over the period between the fourth and the fifteenth day. This had been reenforced by the oral administration of dicalcium phosphate ($CaHPO_4$) from the fourth day on. Administration of halibut liver oil with viosterol was started on the fourteenth day.

When the infant was 26 days old, nineteen days after the calcium gluconate was administered, roentgen study showed that this hard mass contained calcium salts, which were spread throughout the muscles of the right thigh. About one month later the tissues had returned to normal from a clinical standpoint, but roentgen examination showed the persistence of a small amount of precipitate near the hip joint.

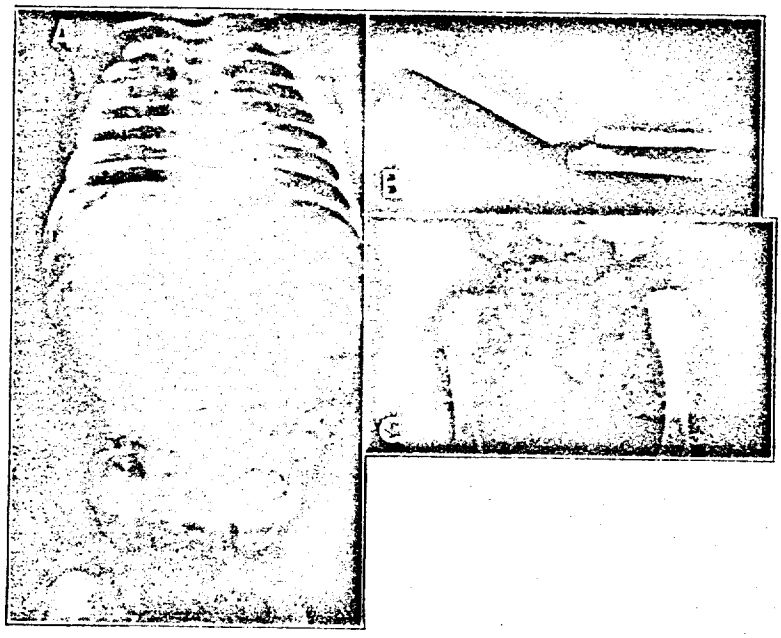

Fig. 2 (case 2).—*A* shows the massive deposits of calcium salts beneath the pleura and within the substance of the right lung and possibly at the base of the left lung. The roentgenogram was taken on the thirty-fifth day of life, thirty days after the last injection of calcium gluconate. *B*, local precipitation of calcium in the left deltoid region. *C*, precipitation of calcium in both gluteal regions. Roentgenograms *B* and *C* were taken forty days after the last injection of calcium gluconate.

At this time it was noticed that the arteries above and below both knee joints could be visualized, and the only possible explanation seemed to be that precipitation of calcium salts had occurred within the arterial coats. Other regions of the body were normal. Two months and one week later this deposit had apparently disappeared, since it could no longer be shown by roentgen study, and at the time of writing the patient, at the age of 4½ years, is an apparently normal boy. Blood calcium values were 6, 8, 13 and 10 mg. per hundred cubic centimeters on the ninth, tenth, eleventh and fourteenth day, respectively.

CASE 2.—The patient, a boy, was born by difficult forceps delivery. A tetany syndrome of extreme degree developed on the second day. As the disease was obstinate, a total of 35 cc. of calcium gluconate was injected intramuscularly over a period of four days and a total of 10 cc. of parathyroid extract (Lilly) within twelve days. One drop of purified vitamin D in propylene glycol (Drisdol) was given in the feeding every three hours from the sixth day on. Oral administration of 10 grains (0.65 Gm.) of calcium lactate every three hours was started on the tenth day. The spinal fluid at the beginning of the fourth day was clear and contained no cellular elements or excess of globulin. The calcium content was 2.2 mg. per hundred cubic centimeters, in spite of the fact that 20 cc. of calcium gluconate and 4 cc. of parathyroid extract had been given within the previous forty-eight hours.

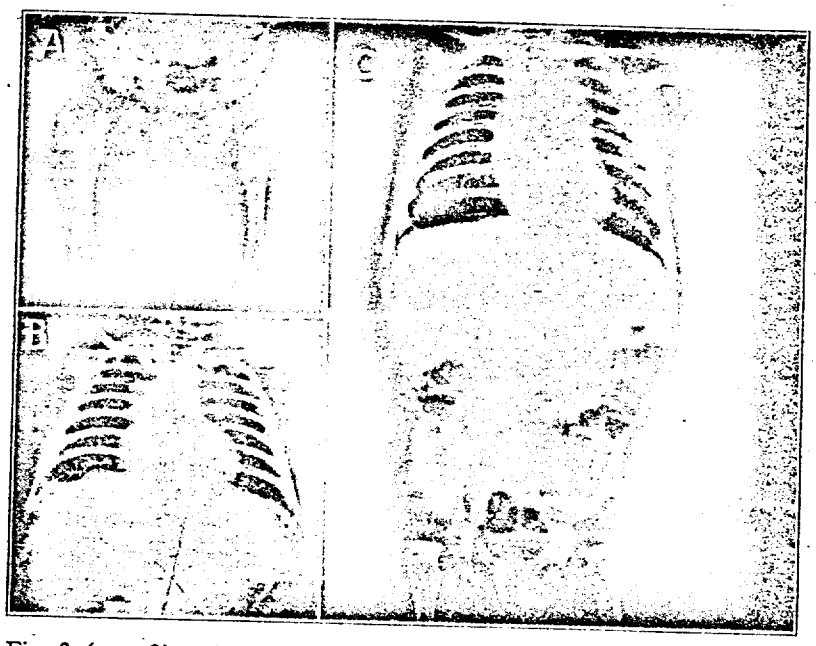

Fig. 3 (case 2).—*A*, partial absorption in the gluteal regions, and *B*, partial absorption in the lungs fifty-eight days after the last injection of calcium gluconate. *C*, complete absorption in all areas at the age of 5½ months. The thymus is markedly enlarged.

As in case 1, at all the sites of injection of calcium the early soft swelling gradually changed to a stony hardness with eventual sloughing. All four extremities were involved, and when the patient was 1 month old roentgen examination demonstrated calcium precipitation in each area.

At the same time the startling observation was made that a rather massive deposit of calcium was present in the substance of the right lung. There were no clinical signs by which its presence could have been recognized. Ten days later this precipitate was essentially unchanged. The blood calcium value was 12.2 mg. The sloughs in the extremities gradually healed, some with the extrusion of a chalky mineral substance. This was identified as calcium phosphate. The calcium deposits, both local and remote, were slowly absorbed, and by the time

the child was 5 months of age they had entirely disappeared. There was never any roentgen evidence of arterial involvement in this case.

COMMENT

The possibility that remote precipitation of calcium will follow the local injection of soluble calcium salts lends an aspect to the problem of treatment which would not be present were the only consideration that of a purely local lesion at the introductory site. While the dangers of local scarring, of sepsis and of other complications merit serious thought, they do not compare in significance with the dangers that might result from the deposition of calcium in arteries, lungs, kidneys or other organs vital to continued existence and development. Fortunately, the process of precipitation is apparently reversible in its action, since in both of these cases absorption ultimately took place.

Tumpeer and Denenholz [2] speculated at some length on the mechanism by which calcium that was injected in the form of the gluconate and the levulinate was precipitated as the phosphate. They felt it to be "possible that this precipitation process reflects the calcium derangement in tetany" and that "whatever the mechanism may be for the production of tetany . . . there is a failure of absorption and ionization from natural or artificial deposits." Their opinion derives added impetus from the recent report of Bakwin,[3] in all of whose cases there was a markedly increased phosphorus content of the blood serum. While it is not my opinion that all newborn infants with tetany must have a low value for blood calcium combined with a high value for phosphorus, Bakwin's work as well as a case reported by Farr [4] showed at least that this is not uncommon. In such instances, perhaps the local deposit of calcium phosphate should not be surprising. The explanation as to why some newborn infants with tetany do and others do not show the local precipitation of calcium salts after the intramuscular injection of calcium gluconate may well center around this very point. A greater theoretic significance, however, is to be found in the application of Bakwin's findings to the remote deposition of calcium salts. If it is this tendency to accumulation of phosphorus in the newborn period that is responsible for the local precipitation of calcium phosphate after the intramuscular injection of the gluconate or the levulinate, then it may well underlie a similar phenomenon at remote portions of the body.

Regardless of whether or not this is the underlying chemical mechanism, it is an undubitable fact that the possibility of a remote deposition of calcium salts greatly complicates the entire subject of the tetany

4. Farr, L. E.: Tetany of the New Born, Yale J. Biol. & Med. 9:333 (March) 1937.

syndrome in the newborn. The manner of its occurrence is purely speculative. The rather massive accumulations that were present in the lung in case 2 were remarkably reminiscent of the localized deposits seen in xanthomatosis. The latter apparently are a result of an active process on the part of the cells of the reticuloendothelial system, by which they retrieve an excess of the lipoid substances present in the circulating fluids of the body. Such a conception is probably rather fantastic in this instance, however, since none of the many aberrant calcifications in the body is known to be a result of cellular activity. A more logical explanation, following the theories of Albright and his associates,[5] assumes that supersaturation of the body fluids with calcium phosphate has occurred. The factor which determines the locality in which such a deposit shall take place may well be some form of cellular injury, as has been suggested by Steck and others[6] in regard to extraosseous calcification that may occur as a result of hypervitaminosis D.

In any event, the possible by-effects are too varied to mention. They would depend on many factors: whether the precipitate was permanent or transient; whether or not the tissues involved were damaged thereby, and finally whether or not such damage, if it occurred, was reparable. So far as my associates and I were able to see in the 2 cases reported here, the calcium salt was eventually reabsorbed. This implies at least that the injury was relatively temporary. It indicates that the blood vessels resumed their original elasticity (if indeed they ever lost it) and that the parenchymatous cells of the liver, pancreas, kidneys and other organs, if they were injured, eventually assumed their normal function. However, according to what is now considered to be fact, such resumption would probably be impossible if the injured cells happened to be those of the central nervous system. Thus permanent damage might have occurred to the brain and /or the spinal cord had calcium been deposited there, even though eventual absorption became complete.

The remote precipitation of calcium salts after treatment of tetany of the newborn brings a new and acute problem into the limelight, namely, what shall be considered as the safe procedure in active treatment. Heretofore it has seemed primarily advisable to raise the level of the blood calcium in the most rapid way. The methods employed have included the injection of such salts as calcium gluconate and calcium levulinate either intramuscularly or intravenously, the feeding of

5. Albright, F.; Bauer, W.; Claflin, D., and Cockrill, J. R.: Studies in Parathyroid Physiology: The Effect of Phosphate Ingestion in Clinical Hyperparathyroidism, J. Clin. Investigation **11**:411 (March) 1932.

6. Steck, I. E.; Deutsch, H.; Reed, C. I., and Struck, H. C.: Further Studies on Intoxication with Vitamin D, Ann. Int. Med. **10**:951 (Jan.) 1937.

any of the usual calcium salts, the oral administration of vitamin D (or its essential equivalent, ultraviolet radiation) and the injection of parathyroid extract. The constant administration of carbon dioxide has seemed important to me, though it seems to have attracted little attention from other writers. Intramuscular injection of calcium gluconate has been shown frequently to result in the local deposit of calcium, often with sloughing of the tissues. This is an undesirable effect which in the cases that have so far come to my attention was not as serious as the condition that was being treated and seemed therefore to be condoned. To overcome this objectionable result, Bakwin advised that this salt be administered only by the intravenous route. Such a procedure has seemed to be an adequate protection against the local lesions that intramuscular injections might initiate, but one wonders whether or not it would be effective in avoiding a remote deposit. Precisely the same question is involved in the oral administration of calcium. Thus the safety of administering calcium in any form becomes a question of paramount importance.

Bakwin treated 2 of his patients with viosterol alone. A third was not treated at all, but that patient showed no active evidence of tetany. In all 4 recovery was prompt, much more so than I can believe would have been the case in any of my series in which the diagnosis was tetany of the newborn. I have seen many newborn infants who had symptoms suggesting this condition get well without treatment of any kind. But in such instances tetany has been considered only as suggested, and the cases add perhaps to my conviction that the condition is of frequent occurrence in newborn though often evanescent. Yet it has been my indisputable experience that usually when administration of viosterol is started there is a temporary increase in the manifestations of tetany in the infant. For this reason it has become my practice to omit the addition of vitamin D until the tetany is largely under control. It therefore becomes highly questionable whether the administration of vitamin D alone can generally be considered as reliably adequate treatment in these cases.

There remains for discussion the use of parathyroid extract. I have felt that this is the most dependable of all the factors important in the treatment of the tetany syndrome in the newborn and have employed it extensively. I cannot agree with many of the more recent writers who have stated that they would restrict the diagnosis of tetany in the newborn to infants with a low level of blood calcium,[7] but from the beginning I have recognized that parathyroid deficiency is probably

7. Bakwin.[3] Farr.[4] Guild, H. G.: Tetany of the New Born, Internat. Clin. **3**:46 (Sept.) 1933. Greenwald, H. M., and Palinsky, M.: Tetany of the New Born, Acta pædiat. **17**:386, 1935.

the principal cause in one class of cases.[8] For this group at least administration of parathyroid extract should be the treatment of choice. That it is extremely effective even in cases in which blood calcium values are normal or but slightly lowered I have had abundant opportunity to observe. Furthermore, in the large number of cases in which no chemical examination has been made, the precise cause of the condition therefore being left in doubt, I have rarely seen it fail to effect a definite, if sometimes transient, improvement.

It is for the very type of patient to whom Bakwin and others would restrict the diagnosis of tetany of the newborn, namely, those in whom there is an obvious parathyroid deficiency, that parathyroid extract should be most effective. Bakwin noted a low value for calcium combined with a high value for phosphorus in the blood of his patients. What could be more effective as a therapeutic agent than a substance the physiologic effect of which is precisely to cause rapid urinary elimination, with lowering of the blood phosphorus level, while at the same time it increases the calcium content in the blood? Since precisely this effect is produced by parathyroid extract,[9] it would seem evident that this substance should be the agent of choice, especially in cases of tetany due to parathyroid deficiency. The necessity of recognizing this fact would seem to be more urgent now that the possible precipitation of calcium salts out of the blood into various tissues of the body has become a reality. In fact, it becomes theoretically questionable whether any other means for raising the calcium level in the blood should be employed until parathyroid extract has been given in doses sufficient to gain active, even if temporary, control of the disease. Such speculation becomes mere guess work, of course, until a much wider experience has been gained with this complication under circumstances which permit accurate and detailed chemical studies.

SUMMARY AND CONCLUSIONS

Two cases are presented in which, complicating the treatment for severe tetany of the newborn, calcium salts were precipitated not only at the site of local injection of calcium gluconate but at remote points in the body.

Reabsorption eventually occurred without known permanent damage.

If this can occur after the local injection of calcium salts, there is no reason to suppose that it could not develop as well after the intravenous or even the oral administration of calcium.

8. Shannon, W. R.: Etiology of the Tetany Syndrome in the New Born, Arch. Pediat. 51:23 (Jan.) 1934.

9. Albright, F., and Ellsworth, R.: Studies on the Physiology of the Parathyroid Glands: I. Calcium and Phosphorous Studies on a Case of Hypoparathyroidism, J. Clin. Investigation 7:183 (June) 1929.

Such a possibility cannot fail to create a feeling of uncertainty as to the safest course to be followed in treatment for tetany of the newborn.

The suggestion is made that, at least for the patients with low calcium values, perhaps parathyroid extract offers the greatest theoretic security against the development of this complication.[10]

It is hoped that the 2 cases reported here will stimulate the interest of other observers in this condition so that an answer to the questions that it raises may be speedily forthcoming.

10. Since this article was written, MacBryde reported on the use of dihydrotachysterol in the treatment of hypoparathyroid tetany. One is inclined to think from his report that this drug might find excellent use in certain cases of tetany of the newborn (MacBryde, C. M.: The Treatment of Hypoparathyroid Tetany with Dihydrotachysterol, J. A. M. A. **110**:767 [March 5] 1938).

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Weaver

COMPARATIVE TOXICOLOGY OF IRON COMPOUNDS

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Gluconate

The great number of iron preparations available for the treatment of iron deficiency anemia is evidence of the incidence and seriousness of the clinical problem and the lack of a universally acceptable preparation for therapy. The principal problems accompanying oral administration of iron preparations involve gastrointestinal distress and, more importantly, serious and even fatal iron toxicities, especially in children.

This report is concerned with the animal toxicities of a new high molecular iron-carbohydrate complex‡ compared to those of several other preparations.

Materials and Methods. Iron-carbohydrate complex represents a ring structure in which the metallic (Fe) ion is sequestered and firmly bound within a polymerized carbohydrate of high molecular weight (30,000). In physical properties, it is a free-flowing, amorphous, brown powder highly soluble in water and is exceptionally high in iron content (approximately 50%). It forms a dark brown colloidal solution that is stable at pH 4 to 11 and to heat.

Other compounds used were exsiccated ferrous sulfate (Fe 29.7%); ferrous gluconate (Fe 11.6%); ferrous fumarate§ (Fe 32.9%); ferric choline citrate (Fe 12.0%); an iron polysaccharide complex||, which contains 20 mg. of trivalent iron per milliliter (given by intravenous injection), and tablets of ferroglycine sulfate complex, available commercially as Ferronord, which were used in pulverized form.

Acute toxicity in mice. The compounds were administered as aqueous solutions where possible, otherwise as fine suspensions. Groups of 10 or more male albino Swiss-Webster mice were given the compounds (Table 1) intravenously (i.v.), intraperitoneally (i.p.), or intragastrically (i.g.). The rate of i.v. injections was 0.01 ml. per second. The animals were observed closely for several hours following injection, and the LD_{50} and 95% confidence limits were determined at the end of 24 hours by the method of Litchfield and Wilcoxon[5]. The animals receiving iron-carbohydrate complex and ferrous sulfate were observed for a period of 7 days following injection and any delayed manifestations of toxicity were recorded. If any deaths occurred after 24 hours, the LD_{50} was recalcu-

TABLE 1.—ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE

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†Present address: Wm. S. Merrell Co., Cincinnati, Ohio.

‡Jefron (Pitman-Moore Co.)

§Kindly supplied by Floyd P. Hallett, Mallinckrodt Chemical Works, St. Louis, Mo.

||Kindly supplied by Dr. A. P. Truant, Astra Pharmaceutical Products, Inc., Worcester, Mass.

TABLE 1.—ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE

Compound	% Fe	No. mice	LD_{50} (19-20 Confidence Limits), mg./kg. i.v. route As salt	As Fe	No. mice	i.p. route As salt	As Fe	No. mice	i.g. route As salt	As Fe
Iron-carbohydrate complex	48.8	40	175 (158–194)	85	30	980 (834–1151)	478.2	30	>8000	>3904
Ferrous sulfate	29.72	55	112 (105–120)	33	105	137 (122–154)	40.7	40	1025 (802–1311)	305
Ferrous gluconate	11.58	55	199 (182–218)	23	30	160 (94–272)	18.5	100	3950 (3543–4404)	457.4
Ferrous fumarate	32.87	—	—	—	40	480 (410–562)	157.8	70	1570 (1353–1821)	516.1
Ferroglycine sulfate complex	15.87	—	—	—	80	365 (341–391)	57.9	50	1940 (1516–2483)	307.9
Ferric choline citrate	12.02	70	210 (192–229)	25	40	151 (120–190)	18.1	40	5500 (4297–7040)	661.1
Iron polysaccharide complex	2.0	30	—	170 (147–197)	50	—	318 (238–426)	—	—	—

rch Center, Pitman-Moore

at is stable at pH 4 to 11

s used were exsiccated fer-
29.7%); ferrous gluconate
s fumarate§ (Fe 32.9%);
e (Fe 12.0%); an iron
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versity Medical Center,

rks, St. Louis, Mo.
s, Inc., Worcester, Mass.

lated at the end of the 7-day observation period. Strain and sex differences in response to iron-carbohydrate complex and ferrous sulfate were evaluated by the use of female albino Swiss-Webster, black female BDF-1 and C-57 and female fawn DBA-2 mice.

ACUTE TOXICITY IN RATS. Male (250 to 350 gm.) and female (150 to 250-gm.) Harlan-Wistar rats in mixed groups of 6 received the iron compounds i.g. in attempts to determine 24-hour LD_{50} in this species.

TOXICITY IN DOGS. Mongrel dogs, unselected as to sex, were used in all acute toxicity studies. Acute, rapid, i.v. injections were made and the LD_{50} determined at 24 hours. In subacute toxicity tests, dogs received iron-carbohydrate complex or ferrous sulfate in gelatin capsules twice daily for approximately one month. The total dose of ferrous sulfate was 0.5 gm. per day (5 dogs) and the total daily doses of iron-carbohydrate complex were 0.5 gm. (3 dogs), 1.0 gm. (3 dogs), and 2.0 gm. (6 dogs). These animals were observed closely for emesis and other outward signs of toxicity. At the end of the test period the dogs were killed (pentobarbital sodium solution intracardially and exsanguination) and subjected to extensive histopathologic studies.

PATHOLOGIC STUDIES. Blood samples from all dogs used in subacute toxicity tests were taken for routine hematologic studies, and thorough necropsy examinations were made for gross lesions.

Fresh specimens of liver, spleen, bone marrow, stomach, small intestine, large intestine, brain, kidneys, adrenal glands, mesenteric lymph nodes, thyroid, and myocardium were fixed in formalin, processed, and sections (about 6 μ) of all tissues were stained with azure-eosin. In addition, sections of liver, spleen, and bone marrow were stained by a modified Gomori's method for iron.

EMETIC STUDIES. The various iron compounds were given in suspension (ferrous sulfate), in solution (iron-carbohydrate complex), or in gelatin capsules. The dogs were not fasted, and no dog was used more than once in these studies.

Results. The results in mice are summarized in Table 1. Since the iron content of the compounds varies considerably comparisons were made on the basis of actual iron content. The iron polysaccharide complex was the least toxic by the i.v. route in mice and iron-carbohydrate complex was next. The other compounds tested were 2 to 4 times as toxic as the iron-carbohydrate complex. The iron-carbohydrate complex was the least toxic of the compounds studied i.p. in mice, though not significantly less toxic than the iron polysaccharide complex. It had only 1/10 the toxicity of ferrous sulfate. Intragastrically, iron-carbohydrate complex was at least 6 times less toxic than any of the other compounds tested. The volumes necessary made it impractical to attempt to determine the i.g. toxicity of the iron polysaccharide complex. There was no significant difference between the 1- and 7-day toxicities for either iron-carbohydrate complex or ferrous sulfate for the i.v. and i.g. routes in mice.

In Swiss-Webster male mice the i.g. LD_{50} was 1025 mg. per kg. for ferrous sulfate. The same dose in female mice of different strains (10 mice in each test) produced the following percentages of deaths: Swiss-Webster, 70; BDF-1, 70; and C-57, 90. The i.g. LD_{50} of iron-carbohydrate complex in male Swiss-Webster was >8000 mg. per kg. The same dose produced no deaths in female Swiss-Webster, BDF-1, C-57 and DBA-2. Thus, there is no evidence of sex or strain differences in these very limited studies.

Results of acute toxicity studies in rats and dogs are summarized in Table 2. None of the compounds are very toxic following i.g. administration to rats and evidently there are only slight differences in the lethal effects of ferrous sulfate, ferrous gluconate and ferroglycine sulfate complex. There was no significant difference between the 1- and 7-day LD_{50} for ferrous sulfate and iron-carbohydrate complex in rats.

Following i.v. administration in dogs, ferric choline citrate and ferrous sulfate were the most toxic of the compounds tested. Ferrous gluconate and

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TABLE 2.—ACUTE TOXICITIES OF IRON COMPOUNDS IN RATS AND DOGS

Compound	% *Fe*	*No. rats*	*i.g. route*		*No. dogs*	*i.v. route*	
			As salt	*As Fe*		*As salt*	*As Fe*
			LD_{50} (*19/20 Confidence Limits*)				
Iron-carbohydrate complex	48.8	10	>8000	>3904	18	94 (78–113)	45.9
Ferrous sulfate	29.7	24	2625 (2323–2966)	780	16	79 (71–88)	23.5
Ferrous gluconate	11.6	24	7460 (6844–8131)	865	9	>400	>46.4
Ferrous fumarate	32.9	24	>7080	>2329	—	—	—
Ferroglycine sulfate complex	15.9	24	5590 (4454–7014)	894	—	—	—
Ferric choline citrate	12.0	12	>8000	960	17	140 (101–190)	16.8
Iron polysaccharide complex	2.0	—	—	—	3	—	>40

iron-carbohydrate complex were the least toxic. The iron polysaccharide complex was given in a dose of 40 mg. per kg. to 3 dogs without lethal effects. Because of the fixed concentration of the solution, the volume necessary for higher dosages was too great to be practical.

weights of 6 dogs on the 2 gm. per day dose of iron-carbohydrate complex showed a 0.1 kg. rise as the maximum change. Emesis occurred once in the 12 dogs during the study with iron-carbohydrate complex and this was at the dose of 0.5 gm. per day. In contrast, emesis occurred 14 times during

TABLE 3.—EMETIC EFFECTS OF IRON COMPOUNDS IN DOGS

Compound	*Form*	*Dose mg./kg.*		*No. Vomiting/ Total No.*	*% Emesis*
		As salt	*As Fe*		
Ferrous sulfate	Suspension	96	28.8 (19.5–42.6)	—	ED_{50}*
	Capsule	62	18.6 (15.3–22.8)	—	ED_{50}
Iron-carbohydrate complex	Solution	600	293	0/4	0
		900	439	0/7	0
	Capsule	300	146	0/2	0
		600	293	1/4	25
		900	439	0/10	0
Ferrous gluconate	Capsule	400	46	2/5	40
		800	93	5/5	100
Ferrous fumarate	Capsule	800	263	4/6	67
Ferroglycine sulfate complex	Capsule	200	32	2/4	50
		400	64	2/2	100
		800	127	2/2	100
Ferric choline citrate	Capsule	800	96	1/5	20
		1200	144	2/6	33

*ED_{50} = Emetic dose for 50% of dogs.

Emetic responses to the various iron compounds in dogs are presented in Table 3. Iron-carbohydrate complex produced less gastrointestinal distress as indicated by emesis than any of the other compounds. Ferrous sulfate and ferroglycine sulfate complex were the most emetic in this study.

In the subacute toxicity studies average weights of dogs given ferrous sulfate and those given iron-carbohydrate complex at the two lower dosage levels decreased the first week and then remained constant or were regained. The changes ranged between 0.6 and 0.8 kg. for the 3 groups. The average

the same period in the 5 dogs given ferrous sulfate. No other gross signs of toxicity were observed.

No gross lesions or microscopic lesions suggestive of iron toxicity were observed in any of the dogs regardless of the compound or dosage level. No significant differences in stained iron content were apparent in the spleen, liver, and bone marrow at any dosage level of either ferrous sulfate or iron-carbohydrate complex. The total red blood cell count and hemoglobin levels were within the normal range in each dog.

Discussion. Studies of ferrous sulfate,

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m. per day. In con-
rred 14 times during

S IN DOGS

No. Vomiting/ Total No.	% Emesis
—	ED_{50}*
—	ED_{50}
0/4	0
0/7	0
0/2	0
1/4	25
0/10	0
	40
5/5	100
4/6	67
2/4	50
2/2	100
2/2	100
1/5	20
2/6	33

in the 5 dogs given
No other gross signs
observed.
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of iron toxicity were
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or dosage level. No
nces in stained iron
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narrow at any dosage
rrous sulfate or iron-
nplex. The total red
and hemoglobin levels
normal range in each

dies of ferrous sulfate,

ferrous gluconate and ferrous fumarate in rodents gave results comparable to those reported by other investigators (Table 4). The consistently lower toxicity observed in our studies for these compounds in rats might be explained by the fact that our studies were done in older animals that had free access to food except during the period of testing; no sex differences were observed in these studies or in the studies conducted in mice.

stained iron content in the spleen, liver, or bone marrow from lower dosages or from ferrous sulfate. This suggests that even high doses of iron-carbohydrate complex may be tolerated without serious side effects.

Summary. 1. Studies in mice indicate that iron-carbohydrate complex is less toxic than ferrous sulfate, ferrous gluconate, ferrous fumarate, ferroglycine sulfate complex, ferric choline citrate and iron polysaccharide complex by the oral and intraperitoneal routes. The iron polysaccharide complex by the intravenous route was the least toxic and iron-carbohydrate complex was next least toxic.

2. None of the compounds tested was less toxic than iron-carbohydrate complex by the oral route in rats or the intravenous route in dogs.

3. Iron-carbohydrate complex produced the least gastrointestinal irritation as indicated by emesis in the dog.

4. Doses of iron-carbohydrate complex equivalent to 1 gm. elemental iron per day for a month failed to produce local irritation or systemic alterations in dogs.

TABLE 4.—ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE AND RATS

Compound	LD_{50}, mg. Fe/kg. mice, i.g.	mice i.v.	rats, i.g.	Reference
Ferrous sulfate	305	33	780	
	306	13	298	Hoppe *et al.*[3]
	230	11	344	Berenbaum *et al.*[1]
				Nissim[6]
	900	14		Keith[4]
				Somers[7]
				Edge *et al.*[2]
Ferrous gluconate	457	23	865	
	429	13	518	Hoppe *et al.*[3]
	320			Berenbaum *et al.*[1]
Ferrous fumarate	516		>2329	
	630		580	Berenbaum *et al.*[1]

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SUMMARIO IN INTERLINGUA

Toxicologia Comparative de Compositos de Ferro

1. Studios in muses indica que—in administrationes per via oral e per via intraperitonee—complexo de ferro e hydrato de carbon es minus toxic que sulfato ferrose, gluconato ferrose, fumarato ferrose, complexo de ferroglycina e sulfato, ferric citrato de cholina, e complexo de ferro e polysaccharido. In administrationes per via intravenose, complexo de ferro e polysaccharido esseva le minus toxic, sequite per complexo de ferro a hydrato de carbon.

2. Nulle del compositos testate esseva minus toxic que complexo de ferro e hydrato de carbon per via oral in rattos o per via intravenose in canes.

3. Complexo de ferro e hydrato de carbon produceva le minus marcate grado de irritation gastrointestinal, a judicar per le emesis provocate in canes.

4. Doses de complexo de ferro e hydrato de carbon equivalente a 1 g de ferro elementic per die durante un mense non produceva irritation local o alterationes systemic in canes.

SUMMARIO IN INTERLINGUA

(See page 309 for original article)

Le Susceptibilitate de Bacillos de Species de Proteus e Providence pro 10 Agentes Antibacterial

Per medio de un meticulosemente standardisate methodo de dilution a tubos, 95 isolatos clinic de *Proteus* (representative del usual distribution de species in le Statos Unite) e 12 isolatos clinic de bacillos del gruppo Providence esseva testate relative a lor susceptibilitate pro 10 agentes anti-infectiose. Omne agente esseva testate a un numero de concentrationes. Tamen, le efficacia de omne le agentes individual esseva judicate a un concentration experimental correspondente al concentration sanguinee que es attingibile in le patientes.

Penicillina esseva le agente le plus efficace contra *Proteus mirabilis*. Kanamycina esseva le agente le plus efficace contra *P. rettgeri, P. morgani, P. vulgaris,* e bacillos del gruppo Providence.

47

than 20 parts per million (0.002

million (0.001 percent).
.. Passes test.

rams, accurately weighed, into a
l. of water, and add 0.5 ml. of
fer solution (350 ml. of glacial
sodium acetate diluted to 1000
thorium nitrate to a sharp color
l. of 0.1 *M* thorium nitrate is
Na_2O_8.
ine by the *Potentiometric Method*,

ner *Titrimetric Method*, page 977.
directed for organic com-
pe. ic acid instead of 30 per-
osition of the sample. The re-
of the *Arsenic Test*, page 865.
a 1-gram sample as directed in
, page 920, using 20 mcg. of lead

directed for organic compounds
oric acid instead of 30 percent
n of the sample. The resulting
Lead Limit Test, page 929.
. Transfer a 1-gram sample,
and dissolve it in 5 ml. of water.
by dissolving 67.5 grams of am-
adding 570 ml. of stronger am-
1000 ml. Then to the buffered
ck T.S., and titrate with 0.1 *M*
of a deep wine-red color. Not

il-closed containers.
ve; sequestrant.

CALCIUM GLUCONATE

$[CH_2OH(CHOH)_4.COO]_2Ca$

$C_{12}H_{22}CaO_{14}$ Mol. wt. 430.38

DESCRIPTION

White, crystalline granules or powder. It is odorless, tasteless, and stable in air. Its solutions are neutral to litmus. One gram dissolves slowly in about 30 ml. of water at 25° and in about 5 ml. of boiling water. It is insoluble in alcohol and in many other organic solvents.

IDENTIFICATION

A. A 1 in 50 solution gives positive tests for *Calcium*, page 926.

B. Place 500 mg. in a test tube and dissolve it in 5 ml. of water by warming. To the warm solution add about 0.7 ml. of glacial acetic acid and 1 ml. of freshly distilled phenylhydrazine, heat on a steam bath for 30 minutes, and allow to cool. Induce crystallization by scratching the inner surface of the tube with a glass rod. Crystals of gluconic acid phenylhydrazide form.

SPECIFICATIONS

Assay. Not less than 98.0 percent and not more than the equivalent of 102.0 percent of $C_{12}H_{22}CaO_{14}$ after drying.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on drying. Not more than 3 percent.

Sucrose and reducing sugars. Passes test.

TESTS

Assay. Dissolve about 800 mg., previously dried at 105° for 16 hours and accurately weighed, in 100 ml. of water containing 2 ml. of diluted hydrochloric acid T.S. While stirring, preferably with a magnetic stirrer, add about 30 ml. of 0.05 *M* disodium ethylenediaminetetraacetate from a 50-ml. buret, then add 15 ml. of sodium hydroxide T.S. and 300 mg. of hydroxy naphthol blue indicator, and continue the titration to a blue end-point. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 21.52 mg. of $C_{12}H_{22}CaO_{14}$.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865, substituting nitric acid for hydrogen peroxide in the wet digestion of the sample.

Heavy metals. Mix a 1-gram sample with 4 ml. of 1 *N* hydrochloric acid, dilute to 25 ml. with water, warm gently until dissolved, and cool. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on drying, page 931. Dry at 105° for 16 hours.

Sucrose and reducing sugars. Dissolve 500 mg. in 10 ml. of hot water, add 2 ml. of diluted hydrochloric acid T.S., boil for about 2 minutes, and cool. Add 5 ml. of sodium carbonate T.S., allow to stand for 5 minutes, dilute with water to 20 ml., and filter. Add 5 ml. of the clear filtrate to about 2 ml. of alkaline cupric tartrate T.S., and boil for 1 minute. No red precipitate is formed immediately.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Miscellaneous and general purpose; buffer; firming agent; sequestrant.

CALCIUM GLYCEROPHOSPHATE

$C_3H_7CaO_6P$

Mol. wt. 210.14

DESCRIPTION

A fine, white, odorless, almost tasteless powder. It is somewhat hygroscopic. One gram dissolves in about 50 ml. of water at 25°. It is more soluble in water at a lower temperature, and citric acid increases its solubility in water. It is insoluble in alcohol.

IDENTIFICATION

A. A saturated solution gives positive tests for *Calcium*, page 926.

B. Heat a mixture of 100 mg. of the sample with 500 mg. of potassium bisulfate. Pungent vapors of acrolein are evolved.

SPECIFICATIONS

Assay. Not less than 98.0 percent of $C_3H_7CaO_6P$ after drying.

Limits of Impurities

Alkalinity. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on drying. Not more than 12 percent.

TESTS

Assay. Weigh accurat[ely] a for 4 hours, and dissolve in 100 chloric acid T.S. Transfer the dilute to volume with water, ar tion into a suitable container, a preferably with a magnetic stir ethylenediaminetetraacetate fr sodium hydroxide T.S. and 300 and continue the titration to disodium ethylenediaminetetra $C_3H_7CaO_6P$.

Alkalinity. A solution of 1 more than 1.5 ml. of 0.1 *N* sulfu of phenolphthalein T.S. as indic

Arsenic. A *Sample Solution* pounds meets the requirements

Heavy metals. Dissolve 50 and dilute to 25 ml. with water. of the *Heavy Metals Test*, page control (*Solution A*).

Lead. A *Sample Solution* pre meets the requirements of the *L* lead ion (Pb) in the control.

Loss on drying, page 931.

Packaging and storage. Sto

Functional use in food N

CALCIU

Sl

$Ca(OH)_2$

DESCRIPTION

A white powder, possessing gram dissolves in 630 ml. of w water. It is soluble in glycerin but is insoluble in alcohol.

IDENTIFICATION

A. When mixed with from 3 smooth magma. The clear, su alkaline to litmus.

characteristic aroma of cognac.
mineral oil. It is very slightly
soluble in glycerin.

l +2°.

: 1.430 at 20°.

0.870.

d in the general method, page

100-mm. tube as directed under

1 the general method, page 897,
d.

m with an Abbé or other re-

directed in the general method,
of 80 percent alcohol.
reliable method (see page 5).

tight containers in a cool place

agent.

OIL

stillation of copaiba balsam, an
1 American species of *Copaifera*
:less to slightly yellow liquid
iba balsam, and an aromatic,
soluble in alcohol, in most fixed
ie in glycerin and practically

l −33°.
1.500 at 20°.
0.907

Limits of Impurities

Gurjun oil. Passes test.

TESTS

Angular rotation. Determine in a 100-mm. tube as directed under *Optical Rotation*, page 939.

Refractive index, page 945. Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific gravity. Determine by any reliable method (see page 5).

Gurjun oil. Add 5 to 6 drops of the sample to 10 ml. of glacial acetic acid containing 5 drops of nitric acid. No purple color develops in 2 minutes, indicating the absence of gurjun oil.

Packaging and storage. Store in full, tight, preferably glass, tin or other suitably lined, or aluminum containers in a cool place protected from light.

Functional use in foods. Flavoring agent.

COPPER GLUCONATE

$[CH_2OH(CHOH)_4COO]_2Cu$

$C_{12}H_{22}CuO_{14}$ Mol. wt. 453.84

DESCRIPTION

A fine, light blue powder. It is very soluble in water and is very slightly soluble in alcohol.

IDENTIFICATION

A. A 1 in 20 solution gives positive tests for *Copper*, page 926.

B. To 5 ml. of a warm solution (1 in 10) add 0.7 ml. of glacial acetic acid and 1 ml. of freshly distilled phenylhydrazine, heat on a steam bath for 30 minutes, and allow to cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

SPECIFICATIONS

Assay. Not less than 98.0 percent and not more than the equivalent of 102.0 percent of $C_{12}H_{22}CuO_{14}$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Reducing substances. Not more than 1 percent.

TESTS

Assay. Dissolve about 1.5 grams, accurately weighed, in 100 ml.

of water in a 250-ml. Erlenmeyer flask, add 2 ml. of glacial acetic acid and 5 grams of potassium iodide, mix well, and titrate with 0.1 *N* sodium thiosulfate to a light yellow color. Add 2 grams of ammonium thiocyanate, mix, then add 3 ml. of starch T.S. and continue titrating to a milk-white end-point. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 45.38 mg. of $C_{12}H_{22}CuO_{14}$.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Lead. A solution of 1 gram in 25 ml. of water meets the requirements of the *Lead Limit Test*, page 929.

Reducing substances. Transfer about 1 gram of the sample, accurately weighed, into a 250-ml. Erlenmeyer flask, dissolve in 10 ml. of water, add 25 ml. of alkaline cupric citrate T.S., and cover the flask with a small beaker. Boil gently for exactly 5 minutes and cool rapidly to room temperature. Add 25 ml. of a 1 in 10 solution of acetic acid, 10.0 ml. of 0.1 *N* iodine, 10 ml. of diluted hydrochloric acid T. S., and 3 ml. of starch T.S., and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in mg., of reducing substances (as D-glucose) by the formula $(V_1N_1 - V_2N_2)27$, in which V_1 and N_1 are the volume and normality, respectively, of the iodine solution, V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution, and 27 is an empirically determined equivalence factor for D-glucose.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

CORIANDER OIL

DESCRIPTION

The volatile oil obtained by steam distillation from the dried ripe fruit of *Coriandrum sativum* L. (Fam. *Umbelliferae*). It is a colorless or pale yellow liquid, having the characteristic odor and taste of coriander.

SPECIFICATIONS

Angular rotation. Between +8° and +15°.

Refractive index. Between 1.462 and 1.472 at 20°.

Solubility in alcohol. Passes test.

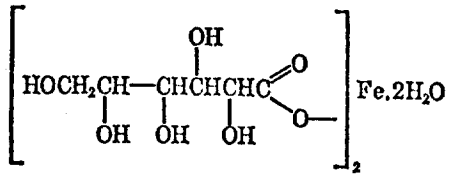
Specific gravity. Between 0.863 and 0.875.

TESTS

Angular rotation. Determine in a 100-mm. tube as directed under *Optical Rotation*, page 939.

Packaging and storage. Store in well-closed containers.
Functional use in foods. Nutrient; dietary supplement.

FERROUS GLUCONATE



$C_{12}H_{22}FeO_{14}.2H_2O$ Mol. wt. 482.18

DESCRIPTION

Fine yellowish gray or pale greenish yellow powder or granules having a slight odor resembling that of burned sugar. One gram dissolves in about 10 ml. of water with slight heating. It is practically insoluble in alcohol. A 1 in 20 solution is acid to litmus.

IDENTIFICATION

A. To 5 ml. of a warm 1 in 10 solution of the sample add 0.65 ml. of glacial acetic acid and 1 ml. of freshly distilled phenylhydrazine, and heat the mixture on a steam bath for 30 minutes. Cool, and scratch the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

B. A 1 in 20 solution gives positive tests for *Ferrous salts*, page 927.

SPECIFICATIONS

Assay. Not less than 95.0 percent of $C_{12}H_{22}FeO_{14}$, calculated on the dried basis.

Loss on drying. Between 6.5 and 10 percent.

Limits of Impurities

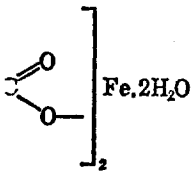
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Chloride. Not more than 700 parts per million (0.07 percent).
Ferric iron. Not more than 2 percent.
Lead. Not more than 10 parts per million (0.001 percent).
Mercury. Not more than 3 parts per million (0.0003 percent).
Oxalic acid. Passes test.
Reducing sugars. Passes test.
Sulfate. Not more than 0.1 percent.

TESTS

Assay. Dissolve about 1.5 grams, accurately weighed, in a mixture

vell-closed containers.
z; dietary supplement.

JCONATE



Mol. wt. 482.18

n yellow powder or granules hav-
rr igar. One gram dissolves
t. It is practically insoluble
to litmus.

zion of the sample add 0.65 ml. of
y distilled phenylhydrazine, and
30 minutes. Cool, and scratch
a glass stirring rod. Crystals of

e tests for *Ferrous salts*, page 927.

of $C_{12}H_{22}FeO_{14}$, calculated on the

10 percent.

3 parts per million (0.0003 per-

rts per million (0.07 percent).
rcent.
r million (0.001 percent).
per million (0.0003 percent).

ent.

ac tely weighed, in a mixture

of 75 ml. of water and 15 ml. of diluted sulfuric acid T.S. in a 300-ml. Erlenmeyer flask, and add 250 mg. of zinc dust. Close the flask with a stopper containing a Bunsen valve, allow to stand at room temperature for 20 minutes, then filter through a Gooch crucible containing an asbestos mat coated with a thin layer of zinc dust, and wash the crucible and contents with 10 ml. of diluted sulfuric acid T.S., followed by 10 ml. of water. Add orthophenanthroline T.S., and titrate the filtrate in the suction flask immediately with 0.1 *N* ceric sulfate. Perform a blank determination (see page 2), and make any necessary correction. Each ml. of 0.1 *N* ceric sulfate is equivalent to 44.62 mg. of $C_{12}H_{22}FeO_{14}$.

Loss on drying, page 931. Dry at 105° for 4 hours.

Arsenic. Place 2 grams of the sample in a 100-ml. round bottom flask fitted with a 24/40 standard taper joint. Add 40 ml. of dilute sulfuric acid (1 in 4) and 2 ml. of 30 percent potassium bromide solution, and connect immediately to a modified Bethge distillation apparatus (see Fig. 3, page 866), or other suitable apparatus having a reservoir with a water jacket which is cooled with ice water. Heat the flask over an Argand burner until the sample dissolves, and collect 25 ml. of distillate. Transfer the distillate to an arsine generator flask, and wash the condenser and reservoir several times with small portions of water. Add bromine T.S. until the distillate is slightly yellow, dilute to 35 ml. with water, and continue as directed in the *Procedure* under *Arsenic Test*, page 865, using 6.0 ml. of *Standard Arsenic Solution* in the preparation of the standard.

Chloride, page 879. Dissolve 1 gram in 100 ml. of water. Any turbidity produced by a 10-ml. portion of this solution does not exceed that shown in a control containing 70 mcg. of chloride ion (Cl).

Ferric iron. Dissolve about 5 grams, accurately weighed, in a mixture of 100 ml. of water and 10 ml. of hydrochloric acid in a 250-ml. glass-stoppered flask, add 3 grams of potassium iodide, shake well, and allow to stand in the dark for 5 minutes. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch T.S. as the indicator. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg. of ferric iron.

Lead. Determine as directed in the test for *Lead* under *Ferrous Fumarate*, page 313.

Mercury. Determine as directed in the test for *Mercury* under *Ferrous Fumarate*, page 313.

Oxalic acid. Dissolve 1 gram in 10 ml. of water, add 2 ml. of hydrochloric acid, transfer to a separator, and extract successively with 50 and 20 ml. of ether. Combine the ether extracts, add 10 ml. of water, and evaporate the ether on a steam bath. Add 1 drop of acetic acid (36 percent) and 1 ml. of calcium acetate solution (1 in 20). No turbidity is produced within 5 minutes.

Reducing sugars. Dissolve 500 mg. in 10 ml. of water, warm, and make the solution alkaline with 1 ml. of ammonia T.S. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the mixture to stand for 30 minutes to coagulate the precipitate. Filter, and wash the precipitate with two successive 5-ml. portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 ml. of diluted hydrochloric acid T.S. in excess. Boil the solution until the vapors no longer darken lead acetate paper, and continue to boil, if necessary, until it has been concentrated to about 10 ml. Cool, add 5 ml. of sodium carbonate T.S. and 20 ml. of water, filter, and adjust the volume of the filtrate to 100 ml. To 5 ml. of the filtrate add 2 ml. of alkaline cupric tartrate T.S., and boil for 1 minute. No red precipitate is formed within 1 minute.

Sulfate, page 879. Any turbidity produced by a 200-mg. sample does not exceed that shown in a control containing 200 mcg. of sulfate (SO_4).

Packaging and storage. Store in tight containers.

Functional use in foods. Nutrient; dietary supplement; coloring adjunct.

FERROUS SULFATE

$FeSO_4 . 7H_2O$ Mol. wt. 278.01

DESCRIPTION

Pale, bluish green crystals or granules. It is odorless, has a saline, styptic taste, and is efflorescent in dry air. In moist air it oxidizes readily to form brownish yellow basic ferric sulfate. Its 1 in 10 solution is acid to litmus, having a pH of about 3.7, and gives positive tests for *Ferrous salts*, page 927, and for *Sulfate*, page 928. One gram dissolves in 1.5 ml. of water at 25° and in 0.5 ml. of boiling water. It is insoluble in alcohol.

SPECIFICATIONS

Assay. Not less than 99.5 percent and not more than the equivalent of 104.5 percent of $FeSO_4 . 7H_2O$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Mercury. Not more than 3 parts per million (0.0003 percent).

TESTS

Assay. Dissolve about 1 gram, accurately weighed, in a mixture of

d not more than the equivalent

en 4.0 and 6.0.

3 parts per million (0.0003

than 10 parts per million (0.001

an 50 parts per million (0.005

million (0.0005 percent).

sulfide. Not more than 0.2

per million (0.005 percent).

ccu y weighed, into a 250-ml.
ute volume with water, and
into a 400-ml. beaker, and add
lamine hydrochloride, 25 ml. of
acetate measured from a buret,
de buffer T.S., and 5 drops of
ution to between 55° and 65°,
end-point. Each ml. of 0.05 *M*
e is equivalent to 9.896 mg. of

determine by the *Potentiometric*

35 ml. of water meets the re-
865.

rams in 25 ml. of water meets the
, page 920, using 20 mcg. of lead

t 20 grams, accurately weighed,
nd on a steam bath for 1 hour.
crucible, wash thoroughly with
and weigh.

l. of water, add 1 ml. of hydro-
h water. Add about 40 mg. of
ml. of ammonium thiocyanate
exceed that produced by 1.0 ml.
in an equal volume of a solution
nts used in the test.

si c. Dissolve 2.0 grams in
mmonium hydroxide, heat to 80°,

and pass hydrogen sulfide through the solution to completely precipitate the manganese. Dilute to 100 ml., mix, and allow the precipitate to settle. Decant the supernatant liquid through a filter, and evaporate 50 ml. of the filtrate to dryness in a tared dish. Add 0.5 ml. of sulfuric acid, ignite to constant weight, cool, and weigh.

Sulfate. Dissolve 10.0 grams in 100 ml. of water, add 1 ml. of diluted hydrochloric acid T.S., mix, and filter. Heat to boiling, then add 10 ml. of barium chloride T.S., and allow to stand overnight. Filter out any precipitate in a tared crucible, wash, ignite gently, cool, and weigh. The weight of the ignited precipitate should not be more than 1.2 mg. greater than the weight obtained in a complete blank test.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

MANGANESE GLUCONATE

$[CH_2OH(CHOH)_4COO]_2Mn.3H_2O$

$C_{12}H_{22}MnO_{14}.2H_2O$ Mol. wt. 481.27

DESCRIPTION

A slightly pink colored powder. It is very soluble in hot water and is very slightly soluble in alcohol.

IDENTIFICATION

A. A 1 in 20 solution gives positive tests for *Manganese*, page 927.

B. It meets the requirements of *Identification test B* under *Copper Gluconate*, page 219.

SPECIFICATIONS

Assay. Not less than 98.0 percent of $C_{12}H_{22}MnO_{14}.2H_2O$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Reducing substances. Not more than 0.5 percent.

TESTS

Assay. Dissolve about 600 mg., accurately weighed, in 50 ml. of water in a 250-ml. porcelain casserole, add 1 gram of hydroxylamine hydrochloride, 10 ml. of ammonia-ammonium chloride buffer T.S., and 5 drops of eriochrome black T.S., and titrate with 0.05 *M* disodium

ethylenediaminetetraacetate to a deep blue color. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 24.06 mg. of $C_{12}H_{22}MnO_{14}.2H_2O$.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 500 mg. in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 25 ml. of water meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Reducing substances. Determine as directed in the test for *Reducing Substances* under *Copper Gluconate*, page 219.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

MANGANESE GLYCEROPHOSPHATE

$C_3H_7MnO_6P.xH_2O$ Mol. wt. (anhydrous) 225.00

DESCRIPTION

A white or pinkish white powder. It is odorless and is nearly tasteless. One gram dissolves in about 5 ml. of citric acid solution (1 in 4). It is slightly soluble in water, and is insoluble in alcohol.

IDENTIFICATION

A. A 1 in 20 solution in diluted hydrochloric acid T.S. gives positive tests for *Manganese*, page 927.

B. Heat a mixture of 100 mg. of the sample with 500 mg. of potassium bisulfate. Pungent vapors of acrolein are evolved.

SPECIFICATIONS

Assay. Not less than 98.0 percent of $C_3H_7MnO_6P$ after drying.

Loss on drying. Not more than 12 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

TESTS

Assay. Dissolve about 1 gram, previously dried at 110° to constant weight and accurately weighed, in 1.5 ml. of nitric acid and 5 ml. of

Free moisture. Heat 20 grams of the sample at 105° for 6 hours, cool in a desiccator, and weigh. Grind the dried sample rapidly, then heat 3 grams of the powder to constant weight at 105°, and calculate the total water content (W). Calculate the percent of free moisture in the sample by the formula $W - 0.3721A$, in which A is the percent of $Na_4Fe(CN)_6.10H_2O$ found in the *Assay*.

Insoluble matter. Dissolve 50 grams of the sample in 300 ml of hot water, and filter off the insoluble matter on a tared Gooch crucible. Wash the residue thoroughly with hot water, dry the crucible in an oven at 105°, cool in a desiccator, and weigh.

Sulfate, page 879. Any turbidity produced by a 500-mg. sample does not exceed that shown in a control containing 350 mcg. of sulfate (SO_4).

Packaging and storage. Store in tight containers.

Functional use in foods. Anticaking agent for sodium chloride.

SODIUM GLUCONATE

$CH_2OH(CHOH)_4COONa$

$C_6H_{11}NaO_7$ Mol. wt. 218.14

DESCRIPTION

A white to tan, granular to fine, crystalline powder. It is very soluble in water and is sparingly soluble in alcohol. It is insoluble in ether.

IDENTIFICATION

A. A 1 in 20 solution gives positive tests for *Sodium*, page 928.

B. To 5 ml. of a warm solution (1 in 10) add 0.7 ml. of glacial acetic acid and 1 ml. of freshly distilled phenylhydrazine, heat on a steam bath for 30 minutes, and cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

SPECIFICATIONS

Assay. Not less than 98.0 percent of $C_6H_{11}NaO_7$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Reducing substances. Not more than 0.5 percent.

of the sample at 105° for 6 hours,
nd the dried sample rapidly, then
ant weight at 105°, and calculate
liate the percent of free moisture
3721*A*, in which *A* is the percent
Assay.
grams of the sample in 300 ml.
oluble matter on a tared Gooch
y with hot water, dry the crucible
or, and weigh.
y produced by a 500-mg. sample
rol containing 350 mcg. of sulfate

ight containers.
ing agent for sodium chloride.

JCONATE

H)$_4$COONa

Mol. wt. 218.14

rstalline powder. It is very solu-
e in alcohol. It is insoluble in

ve tests for *Sodium*, page 928.
n 10) add 0.7 ml. of glacial acetic
enylhydrazine, heat on a steam
ice crystallization by scratching
h a glass stirring rod. Crystals
n.

of $C_6H_{11}NaO_7$.

. 3 parts per million (0.0003 per-

e than 20 parts per million (0.002

n (0.001 percent).
e than 0.5 percent.

TESTS

Assay. Transfer about 150 mg., accurately weighed, into a clean, dry 200-ml. Erlenmeyer flask, add 75 ml. of glacial acetic acid and dissolve by heating on a hot plate. Cool, add quinaldine red T.S., and titrate with 0.1 *N* perchloric acid in glacial acetic acid, using a 10-ml. microburet, to a colorless end-point. Each ml. of 0.1 *N* perchloric acid is equivalent to 21.81 mg. of $C_6H_{11}NaO_7$.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 1 gram in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 25 ml. of water meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Reducing substances. Determine as directed in the test for *Reducing substances* under *Copper Gluconate*, page 219.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement; sequestrant.

SODIUM HYDROXIDE

Caustic Soda

NaOH Mol. wt. 40.00

DESCRIPTION

White, or nearly white, pellets, flakes, sticks, fused masses, or other forms. Upon exposure to air, it readily absorbs carbon dioxide and moisture. One gram dissolves in 1 ml. of water. It is freely soluble in alcohol. A 1 in 25 solution gives positive tests for *Sodium*, page 928.

SPECIFICATIONS

Assay. Not less than 95.0 percent of total alkali, calculated as NaOH.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Carbonate (as Na_2CO_3). Not more than 3 percent.
Heavy metals (as Pb). Not more than 30 parts per million (0.003 percent).
Insoluble substances and organic matter. Passes test.
Lead. Not more than 10 parts per million (0.001 percent).
Mercury. Not more than 1 part per million (0.0001 percent).

tetrasodium; tetrasodium
rsene; Sequestrene; Tetrine;
: Nullapon; Aquamollin;
alsol; Syntes 12a; Tyclaro-
$CCH_2)_2NCH_2CH_2N(CH_2$-
$H_{12}N_2Na_4O_8$; C 31.59%,
O 33.67%. Prepn: Bers-
to Martin Dennis Co.);
(1949). For bibliography
Chelation" issued by The
ch.
.9 lb/gallon Very sol in
of 1% soln 11.3. Reacts
metallic ions forming sol
salt is more sol in alcohol

gram complexes 215 mg
maceuticals in the form of
prevent calcium-depleting

calcium removal.

ethylate; caustic alcohol.
29%, H 7.41%, O 23.51%.

powder. Dec on exposure
eping. Dec by water into
decompn, in abs alcohol.
light and in a cool place.

sulfovinate. $NaC_2H_5SO_4$.-
alt 89.15%, H_2O 10.84%,
S 19.30%, O 48.15%, abs
75.92%, SO_4 58.82%.
Soluble in 0.7 part water,

hexacyanoferrate(III).
Anhydr salt 93.97%.
2.22%. C_6FeN_6-
88%, N 29.92%.

Soluble in 5.5 parts cold
p well closed.

hexacyanoferrate(II);
prussiate yellow. Na_4-
Anhydr salt 62.79%,
4%, CN 32.25%. For the
N 27.65%, Na 30.26%.

$_2O$, pale yellow, mono-
s. Steady dehydration
ydr at 81.5°. Dec 435°,
carbon, and nitrogen.
calcd as the anhydr salt);
at 53° = 28.1%; at 85°
ractically insol in most

yanide solns to slightly
ecipitation of insol Prus-
$_4[Fe(CN)_6]_3$. Alkaline
$Fe(C_6$-$)_6$]. Sodium ferro-
s in general. Used in ore
ching, toning, and fixing.
dditive to pickling baths.
stabilizer in welding rod
ion catalyst. *Human*
cal bondage between the
yanides have a low order
ferrocyanide have been
uicides without apparent
n hot or concd acids and
or any length of time to
de. Waste ferrocyanides
exceed 2 ppm because
n.

tetrafluoroborate; sodium
2. Na 20.94%, B 9.85%,
eq $2H_3BO_3 + 8HF +$
O_2; Balz, Wilke-Dorfurt,
1927)
pr[illegible] d^{20} 2.47, mp
ish g[illegible] when absolutely

dry. Solubility in water at 26° = 108 g/100 ml; at 100° = 210 g/100 ml. Sparingly sol in alcohol. Aq solns have a bitter taste and are acid to litmus.

USE: Fluorinating agent, *see* Lawton, Levy, *J. Am. Chem. Soc.* 77, 6083 (1955).

Sodium Fluoride. Villiaumite. Florocid; Flura-Drops; Fluoritadent; Karidium. NaF; mol wt 42.00. Na 54.75%, F 45.24%. Prepd by fusing cryolite with NaOH; by adding equivalent amounts of NaOH or Na_2CO_3 to 40% HF. Precipitation is instantaneous and crystal size depends on pH, but too much HF yields sodium bifluoride ($NaHF_2$)]: Müller, *Chemiker-Ztg.* 52, 5 (1928). Technical grades are 90% and 95% NaF, light (37 cu in/lb) and dense (23 cu in/lb), and 98%. The impurities are mainly sodium and aluminum fluosilicates.

Cubic or tetragonal crystals (NaCl lattice). d 2.78. mp 993°, bp 1704°. *Poisonous!* Solubility in water at 15° = 4.0 g/100 ml, at 25° = 4.3 g/100 ml, at 100° = 5.0 g/100 ml. Insoluble in alcohol. Aq solns have an alkaline reaction caused by partial hydrolysis. pH of freshly prepd salt soln 7.4. Aq solns etch glass, but the dry crystals or powder may be kept in glass bottles. Sodium fluoride sold as household insecticide must be tinted Nile Blue.

USE: As insecticide, particularly for roaches and ants; in other pesticide formulations; constituent of vitreous enamel and glass mixes; as a steel degassing agent; in electroplating; in fluxes; in heat-treating salt compositions; in the fluoridation of drinking water; for disinfecting fermentation apparatus in breweries and distilleries; preserving wood, pastes and mucilage; manuf of coated paper; frosting glass; in dental laboratories.

MED USE: For prophylaxis of dental caries. Formerly in hyperthyroidism, rheumatoid arthritis, epilepsy. *Dose:* For caries prophylaxis, 0.7 to 1 ppm of drinking water; topically, 2% soln applied directly to teeth. *Human Toxicity:* Severe symptoms from ingestion of 0.25 to 0.45 g. Death from 4 g. *Sublethal:* nausea and vomiting, abdominal distress, diarrhea, stupor, weakness. *Lethal:* muscular weakness, tremors, convulsions, collapse, dyspnea, respiratory and cardiac failure, death. *Chronic:* mottling of tooth enamel, osteosclerosis.

VET USE: Poultry lice; roundworms of swine. *Dose:* swine, 1% in dry feed.

Sodium Folate. *Folic acid sodium salt;* sodium pteroylglutamate; sodium Folvite. $C_{19}H_{18}N_7NaO_6$; mol wt 463.39. C 49.24%, H 3.92%, N 21.16%, O 20.72%, Na 4.96%.

Sold only as sterile soln in ampuls. Clear, mobile liquid. Yellow to orange-yellow color. pH between 8.5 and 11.0. For spectrophotometric data *see* Folic Acid.

MED USE: In folic acid deficiency.

Sodium Formaldehydesulfoxylate. *Hydroxymethanesulfinic acid sodium salt;* formaldehyde sodium sulfoxylate; formaldehydesulfoxylic acid sodium salt; sodium hydroxymethanesulfinate; sodium methanalsulfoxylate; Aldanil; Rongalite; Rongalite C. $Na[HOCH_2SO_2]$; mol wt 118.09. CH_3NaO_3S; C 10.17%, H 2.56%, Na 19.47%, O 40.65%, S 27.16%. Prepn: Heyl, Greer, *Am. J. Pharm.* 94, 80 (1922); Binns, U.S. pat. 2,013,125 (1935 to Virginia Smelting Co.); Postnikov, Kunin, *J. Applied Chem.* (U.S.S.R.) 13, 185 (1940). Structure of dihydrate: Truter, *J. Chem. Soc.* 1955, 3064; 1962, 3400.

Obtained as the dihydrate, $Na[HOCH_2SO_2].2H_2O$, crystals, mp 63-64°, dec at higher temp. Odorless when freshly prepd, but quickly develops a characteristic (garlic) odor. Freely sol in water; practically insol in abs alcohol, ether, benzene. Readily dec by dil acids. Aq soln is practically neutral. *Keep well closed in a cool place.* LD s.c. in mice, 4.0 g/kg: Rosenthal, *Public Health Rept.* (*U.S.*) 49, 908 (1934).

USE: In vat color printing pastes: Borstelmann, Fordemwalt, U.S. pat. 2,597,281 (1952 to Am. Cyanamid Co.). In polymerization of ethylenic compds: Brit. pats. 816,252 and 852,593 (1959 to Hercules Powder Co. and 1960 to Air Reduction Co.). In manuf of arsphenamines: Krumwiede, *J. Am. Pharm. Assoc.* 8, 795 (1919); Heyl, Miller, *ibid.* 11, 412 (1922).

MED USE: Formerly to treat mercury poisoning. *Human Toxicity:* Very low toxicity. Up to 10 g i.v. is tolerated by humans.

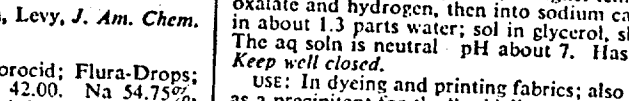
Sodium Formate. HCOONa; mol wt 68.02. $CHNaO_2$; C 17.66%, H 1.48%, O 47.05%, Na 33.81%, formic acid 67.67%.

White, deliquesc granules or cryst powder; slight odor of formic acid. d 1.92. mp 253°; at higher temp dec into sodium oxalate and hydrogen, then into sodium carbonate. Soluble in about 1.3 parts water; sol in glycerol, slightly in alcohol. The aq soln is neutral pH about 7. Has buffering action. *Keep well closed.*

USE: In dyeing and printing fabrics; also in anal. chemistry as a precipitant for the "noble" metals. Solubilizes trivalent metal ions in soln by forming complex ions. Buffering action adjusts the pH of strong mineral acids to higher values.

MED USE: Has been used as caustic, astringent.

Sodium Gluconate. *Gluconic acid sodium salt.* C_6H_{11}-NaO_7; mol wt 218.13. C 33.04%, H 5.08%, Na 10.54%, O 51.34%. The normal sodium salt of gluconic acid.



Crystals. The technical grade may have a pleasant odor. Solubility in water at 25° = 59 g/100 ml. Sparingly sol in alcohol. Insoluble in ether. Aq solns are stable to short boiling periods.

USE: As sequestering agent forming water-sol complexes with calcium in alkaline media and with iron in near neutral solns. Used in metal plating, mineral tanning of hides, mordanting fabrics, and in water-paste paints. Has been suggested as a photographic processing aid.

Sodium Glutamate. *Glutamic acid sodium salt;* monosodium glutamate; Ajinomoto; Glutacyl; RL-50; Vetsin; Chinese seasoning; MSG; Accent; Zest; Glutavene. The monosodium salt of the naturally occurring L-form of glutamic acid. $HOOCCH(NH_2)CH_2CH_2COONa$; mol wt 169.12. Glutamic acid: 86.98%. $C_5H_8NNaO_4$: C 35.51%, H 4.77%, O 37.84%, N 8.28%, Na 13.60%. Produced by hydrolysis of vegetable proteins (*see also* Glutamic Acid): Ikeda, Suzuki, Brit. pat. 9440; *C.A.* 5, 836 (1910); U.S. pat. 1,015,891; *C.A.* 6, 717 (1912); from Steffens waste from beet-sugar molasses by acid hydrolysis: Ikeda, U.S. pat. 1,721,820; *C.A.* 23, 4591 (1929); *see also* Bartow, Albrook, U.S. pat. 1,992,804; *C.A.* 29, 2548 (1935); Royal, U.S. pat. 2,373,342; *C.A.* 39, 4510 (1945); by alkaline hydrolysis: Masuda, Royal, Marshall, U.S. pat. 1,947,563 (1934 to Larrowe-Suzuki Co.); Shafor *et al.*, U.S. pat. 2,829,161 (1958 to Internat. Minerals). Prepn of cryst Na-glutamate: Shildneck, U.S. pat. 2,306,646; *C.A.* 37, 3107 (1943). As a rule, wheat gluten, corn gluten, and sugar beet products are used in the U.S., while soya bean protein is used in the Orient. Flow sheets and condensed descriptions of mfg methods: Faith, Keyes, Clark, *Industrial Chemicals*, 2nd ed (Wiley, New York, 1957), p 522.

White or almost white, cryst powder. The monohydrate, $C_5H_8NNaO_4.H_2O$, forms needles. Slight peptone-like odor. Meat-like taste. The optimum concn is from 0.2 to 0.5% in normally salted food. NaCl must be present to produce an attractive glutamate taste. A 1% concn or more is liable to produce a sweetish taste. L-Sodium glutamate is slightly levorotatory in water, but dextrorotatory in acid solns (the free L-acid is dextrorotatory). $[\alpha]_D^{25}$ +24.2° to +25.5° (c = 8.0 in 1.0*N* HCl). pH of 0.2% soln = 7.0. Very sol in water; sparingly sol in alcohol.

USE: To impart meat flavor to foods, to enhance other natural food flavors. To improve the taste of tobacco.

MED USE: To reduce blood ammonia levels in ammoniacal azotemia. Has also been used in psychosis and mental retardation. *Dose:* i.v. 29 g in 1000 ml of 5% dextrose soln for hepatic coma.

Sodium Glycerophosphate. $Na_2C_3H_5(OH)_2PO_4.5\frac{1}{2}H_2O$; mol wt 315.15. $C_3H_7Na_2O_6P$. Anhydr salt 68.56%, H_2O 31.44%, glycerophosphoric acid 54.61%, glycerol 29.21%, H_3PO_4 31.10%, P 9.84%, Na 14.59%, C 11.43%, H 5.76%, O 58.39%. The so-called beta form is usually obtained as a solid and is the medicinal form described here. The alpha form is difficult to crystallize and is usually obtained as a syrup. Structure: *see* Glycerophosphoric Acid.

White, odorless, scale-like crystals; dec above 130°. Soluble in about 1.5 parts water; more sol in hot water; insol in alcohol. The aq soln is alkaline. pH about 9.5.

MED USE: Has been used as tonic.

VET USE: Formerly used as a so-called "nervine tonic." *Dose:* dogs: 300 mg.

Ferrosoferric Oxide. Ferric ferrous oxide; triiron tetraoxide; black iron oxide; magnetic iron oxide; Ethiops iron. Fe_3O_4; mol wt 231.55. Fe 72.36%, O 27.64%. Occurs in nature as the mineral *magnetite* (red-black lumps). Prepn: *Gmelin's Handb. anorg. Chem.*, System no. 59 (Iron), part B, pp 36-62 (1932); *Ullmanns Encyklopädie der technischen Chemie*, vol. 6, 420 (1955). *Review:* Robl, *Angew. Chem.* 70, 367 (1958).

Black cubes or amorphous powder. mp 1538°; d 5.2. Oxidized to Fe_2O_3 on heating in air. Practically insol in water; sol in acids.

USE: Pigment in paints, linoleum, ceramic glazes; in coloring glass; as a polishing compd; in the textile industry; in cathodes; as catalyst.

Ferrous Arsenate. $Fe_3(AsO_4)_2$; mol wt 445.37. As 33.64%, Fe 37.62%, O 28.74%. In the article of commerce part of the iron is in a ferric state. Prepn: *Gmelin's Handbook anorg. Chem.*, System no 59 (Iron), part B, p 781 (1932).

Hexahydrate, $Fe_3(AsO_4)_2.6H_2O$, greenish or yellowish-brown amorphous powder. Odorless; tasteless. Practically insol in water; sol in mineral acid.

MED USE: Formerly in chronic skin conditions.

Ferrous Bromide. $FeBr_2$; mol wt 215.68. Br 74.11%, Fe 25.89%. Prepn: Baxter, *Z. Anorg. Allgem. Chem.* 38, 236 (1904); Baxter *et al.*, *ibid.* 70, 333 (1911); Kühnl, Ernst, *ibid.* 317, 84 (1962); Schimmel, *Ber.* 62, 963 (1929).

Light yellow to dark brown hygroscopic crystals. mp 684°. d_4^{25} 4.63. Very sol in water and alcohol. *Keep tightly closed.*

Hexahydrate, $FeBr_2.6H_2O$, pale green to bluish-green, rhombic prisms. Loses $2H_2O$ at 49°, another $2H_2O$ at 83°. Rapidly oxidized in moist air. *Keep tightly closed.*

USE: Polymerization catalyst.

MED USE: Formerly in chorea, tuberculous cervical adenitis.

Ferrous Carbonate Mass. Blaud's mass; Fecarb; Vallet's mass. Contains 36–41% $FeCO_3$, the remainder consisting of honey and sugar. Prepn: *U.S.D.*, 25th ed, p 575.

Dark greenish-gray to brown, moderately soft mass. Practically insol in water; appreciably sol in water satd with CO_2; sol in dil acids.

MED USE: Has been used in iron deficiency anemia.

VET USE: In iron deficiency. *Dose:* for cattle and horses 6 g; for dogs 200–500 mg.

Ferrous Carbonate Saccharated. Freshly pptd $FeCO_3$ protected from oxidation by admixture with sugar. Contains not less than 15% $FeCO_3$. Prepn: *U.S.D.*, 25th ed, p 575.

Olive-gray to greenish-brown powder. Odorless. Partially sol in water, completely sol in dil mineral acids.

MED USE: Has been used in iron deficiency anemia.

VET USE: In iron deficiency. *Dose:* for horses and cattle 8–15 g; for sheep and swine 2–4 g; for dogs 0.3–1.0 g.

Ferrous Chloride. $FeCl_2$; mol wt 126.76. Cl 55.94%, Fe 44.06. Occurs in nature as the mineral *lawrencite*. Prepn: Gayer, Woontner, *Inorg. Syn.* 5, 179, (1957); Kovacic, Brace, *ibid.* 6, 172 (1960); *cidem, J. Am. Chem. Soc.* 76, 5491 (1954); Kangro, Petersen, *Z. Anorg. Allgem. Chem.* 161, 157 (1950); Kühnl, Ernst, *ibid.* 317, 84 (1962).

White rhombohedral crystals; may sometimes have a green tint. Very hygroscopic. mp 674°; bp 1023°; d^{25} 3.16. Can be sublimed in a stream of HCl at about 700°. Forms $FeCl_3$ and Fe_2O_3 on heating in air. Freely sol in water, alcohol, acetone; slightly sol in benzene; practically insol in ether.

Dihydrate, $FeCl_2.2H_2O$, white monoclinic crystals with pale green tint. Loses $1H_2O$ at 120°. Soluble in water.

Tetrahydrate, $FeCl_2.4H_2O$, pale green to blue-green, monoclinic crystals or cryst powder. Loses $2H_2O$ at about 75°. d 1.93. Soluble in water, alcohol. The technical product may not be completely sol without the addn of acid. Aq solns are readily oxidized.

USE: In metallurgy; as reducing agent; in pharmaceutical prepns; as mordant in dyeing. *Human Toxicity:* Mild irritant.

Ferrous Citrate. Several forms of this salt are known. Prepn from citric acid and Fe powder: Oroshnik, Haffcke, U.S. pat. 2,904,573 (1959 to Ortho Pharmaceutical Corp.); and ferrous salts: Carlson, U.S. pat. 3,091,626 (1963 to Scherer Corp.).

$Fe(C_6H_6O_7).H_2O$, *monoferrous acid citrate monohydrate.* Mol wt 267.99; $C_6H_6FeO_7.H_2O$; anhydr salt 93.28, H_2O is 6.72%. For the anhydr salt, C 30.43%, H 2.42%, Fe 22.34%, O 44.81%. Powder. Practically insol in water, alcohol, acetone.

$Fe_3(C_6H_5O_7)_2.10H_2O$, *triferrous dicitrate decahydrate.* Mol wt 725.92; $C_{12}H_{10}Fe_3O_{14}.10H_2O$; anhydr salt 75.18%, H_2O 24.82%. For the anhydr salt, C 26.41%, H 1.85%, Fe 30.70%, O 41.04%. Very slightly colored powder or white crystals. Very stable to air oxidation. If H_2O is removed by vacuum desiccation, the dehydrated ferrous salt rapidly oxidizes to a ferric salt. Practically insol in water, acetone.

MED USE: In iron deficiency states.

Ferrous Fluoride. FeF_2; mol wt 93.85. F 40.49%, Fe 59.51%. Prepn from $FeCl_2$ and HF gas: Kwasnik in *Handbook of Preparative Inorganic Chemistry*, G. Brauer, Ed., (2nd ed, Academic Press, 1963), p 266; from Fe powder and liq HF: Muetterties, Castle, *J. Inorg. Nucl. Chem.* 18, 148 (1961).

Tetragonal crystals (rutile type) or powder. mp >1100°. Sublimes at about 1100°. d 4.09. Sparingly sol in water; more sol in dil HF; practically insol in alcohol, ether, benzene.

USE: As catalyst in organic reactions.

Ferrous Fumarate. Cpiron; Erco-Fer; Feostat; Feroton; Ferrofume; Fersamal; Firon; Fumafer; Fumar F; Ircon; One-Iron; Palafer; Toleron; Tolferain; Tolifer. $FeC_4H_2O_4$; mol wt 169.91. C 28.27%, H 1.19%, O 37.67%, Fe 32.87%. Prepd by mixing hot aq solns of ferrous sulfate and sodium fumarate and separating the resulting slurry by filtration: Bertsch, Lemp, U.S. pat. 2,848,366 (1958 to Mallinckrodt Chemical Works). The hot soln of sodium fumarate is preferably added to the ferrous sulfate soln. The commercial material contains a min of 31.3% total Fe and not less than 2.0% ferric iron.

Reddish-orange to reddish-brown, granular powder. Apparent density: 14 fl oz/lb. Odorless; almost tasteless. Not melted at 280°. Solubility at 25° in water: 0.14 g/100 ml; in alcohol <0.01 g/100 ml. Solubility in acid is limited by liberation of fumaric acid: Up to 0.45 g can be dissolved in 100 ml of 1.0*N* HCl and up to 0.6 g in 0.1*N* HCl.

MED USE: Hematinic in iron deficiency anemia.

Ferrous Gluconate. Fergon; Ferlucon; Ferronicum; Gluco-Ferrum; Iromin[Gador]; Irox; Nionate. $Fe[HOCH_2(CHOH)_4CO_2]_2$; mol wt 446.16. $C_{12}H_{22}FeO_{14}$; C 32.30%, H 4.97%, Fe 12.52%, O 50.21%. Prepn from Ba gluconate and $FeSO_4$: *U.S.D.*, 25th ed, p 576. Prepn of isotonic solns: Hammarlund, *Pharm. Acta Helv.* 35, 593 (1960).

Dihydrate, $Fe[HOCH_2(CHOH)_4CO_2]_2.2H_2O$, yellowish-gray or pale greenish-yellow powder. Slight odor of caramel. Acid to litmus. Soluble in water; practically insol in alcohol. Aq solns are stabilized by the addition of glucose. A suitable flavoring agent consists of about 20% of syrup of orange with 0.3% citric acid. Extensive stability studies on aq solns: Johnson, Thomas, *J. Pharm. & Pharmacol.* 6, 1037 (1954).

MED USE: In iron deficiency anemia. *Dose:* Oral 300 mg. *Side Effects:* G.I. disturbances may occur. *See also* Ferrous Sulfate.

Ferrous Hydroxide. $Fe(OH)_2$; mol wt 89.87. Fe 62.15%, H 2.24%, O 35.61%. Prepn: Rihl, Fricke, *Z. Anorg. Allgem. Chem.* 251, 406 (1943).

White amorphous powder or white to pale green hexagonal crystals. Converted to $Fe(OH)_3$ on exposure to air; may ignite spontaneously on exposure to air if finely divided. Practically insol in water; more sol in solns of NH_4 salts; sol in concd NaOH soln.

Ferrous Iodide. FeI_2; mol wt 309.67. Fe 18.04%, O 81.96%. Prepn: Lux in *Handbook of Preparative Inorganic Chemistry*, G. Brauer, Ed., (2nd ed, Academic Press, 1965), p 1495; Chaigneau, *Bull. Soc. Chim. France* 1957, 886; Lieser, Elias, *Z. Anorg. Allgem. Chem.* 316, 208 (1962).

Large, thin, red-violet crystals or black leaflets. Very hygroscopic. Soluble in water, alcohol, ether; aq soln is readily oxidized by air. *Keep tightly closed and protected from light.*

USE: As catalyst for organic reactions.

MED USE: Formerly in chronic tuberculosis.

VET USE: Source of iron and iodine.

Ferrous Lactate. $Fe(C_3H_5O_3)_2$; mol wt 233.99. $C_6H_{10}FeO_6$; C 30.80%, H 4.31%, Fe 23.87%, O 41.03%.

Powder-blue, turquoise, or royal blue crystals. Soluble in water; practically insol in most organic solvents.

Dihydrate, $Cu(HCOO)_2.2H_2O$, very pale blue, monoclinic needles. Loses $2H_2O$ on standing in air. Soluble in water.

Tetrahydrate, $Cu(HCOO)_2.4H_2O$, large, light-blue, monoclinic, holohedral prisms. Soluble in water; very slightly sol in alcohol; practically insol in most organic solvents.

USE: As antibacterial agent in the treatment of cellulose.

Cupric Gluconate. $Cu[CH_2OH(CHOH)_4CO_2]_2$; mol wt 453.85. $C_{12}H_{22}CuO_{14}$; C 31.76%, H 4.89%, Cu 14.00%, O 49.36%. Prepd from gluconic acid and basic cupric carbonate: Suzuki *et al.*, Jap. pat. 2889 ('63) (to Dainippon Pharmaceutical Co., Ltd.); *C.A.* 59, 11264e (1963).

Hydrate, $Cu[CH_2OH(CHOH)_4CO_2]_2.H_2O$, light blue to bluish-green, odorless crystals or cryst powder. Astringent taste. Solubility in water at 25° = 30 g/100 ml; slightly sol in alc; practically insol in most other organic solvents.

USE: In dietary supplements as a readily assimilable form of copper; as oral deodorant.

Cupric Glycinate. *Bis(glycinato)copper;* cupric aminoacetate; glycine copper complex; glycocoll-copper. $Cu(H_2NCH_2COO)_2$; mol wt 211.66. $C_4H_8CuN_2O_4$; C 22.70%, H 3.81%, Cu 30.02%, N 13.24%, O 30.24%. Prepn from glycine and a cupric salt: Tomita, *Bull. Chem. Soc. Japan* 34, 280 (1960).

Hydrate, $Cu(H_2NCH_2COO)_2.H_2O$, long, deep-blue, rhombic needles. Loses H_2O at 123°, chars at 213°, and dec with gas evolution at 228°. Soluble in water; slightly sol in alcohol.

Dihydrate, $Cu(H_2NCH_2COO)_2.2H_2O$, light blue, powdery crystals. Loses one H_2O at 103°, remaining H_2O at about 140°. Dec with gas evolution at about 225°. Soluble in water.

USE: In photometric analysis for copper.

VET USE: Has been used in copper deficiency in cattle and sheep.

Cupric Hexafluorosilicate. Cupric fluosilicate; cupric silicofluoride. $CuSiF_6$; mol wt 205.93. Cu 30.90%, F 55.44%, Si 13.66%. Prepn: Worthington, Haring, *Ind. Eng. Chem., Anal. Ed.* 3, 7 (1931).

Tetrahydrate, $CuSiF_6.4H_2O$, blue, monoclinic, efflorescent crystals. d_4^{20} 1.62. Readily sol in water. *Keep well closed.*

USE: Dyeing and hardening white marble; treating plant diseases.

Cupric Hydroselenite. $Cu(HSeO_3)_2$; mol wt 319.48. Cu 19.89%, H 0.63%, O 30.05%, Se 49.43%. Prepn: *Gmelin's Handb. anorg. Chem.*, System no. 60 (Copper), part B; 8th ed, p 612 (1958).

Bluish-green microscopic prisms. Loses water when heated in a sealed tube. Dec in water to cupric selenite. Soluble in acids.

Monohydrate, $Cu(HSeO_3)_2.H_2O$, green or blue monoclinic crystals. Loses water at 100°.

Dihydrate, $Cu(HSeO_3)_2.2H_2O$, gray-blue crystals. Practically insol in water.

Trihydrate, $Cu(HSeO_3)_2.3H_2O$, green monoclinic crystals. Effloresces in air. Loses water at 100°. Practically insol in water, but converted to $CuSeO_3$ by boiling water; sol in acids.

Cupric Hydroxide. Copper hydrate; hydrated cupric oxide. $Cu(OH)_2$; mol wt 97.56. Cu 65.13%, H 2.07%, O 32.80%. Commercial prepn: Furness, U.S. pat. 1,800,828 (1931 to Cellosilk Co.); *idem*, U.S. reissue pat. 24, 324 (1957 to Copper Research); Rowe, U.S. pat. 2,536,096 (1951 to Mountain Copper Co., Ltd.); laboratory prepn: Gauthier, *Bull. Soc. Chim. France* 1960, 353.

Blue to blue-green gel or light blue cryst powder. Stability is dependent on the method of prepn; may dec to black CuO on standing a few days or on heating. d 3.37. Practically insol in water; sol in concd alkali when freshly pptd; sol in acids, NH_4OH.

USE: In manuf of rayon, battery electrodes, other Cu salts; as mordant in dyeing; as pigment; in fungicides, insecticides; as feed additive; in treating and staining paper; in prepn of Schweitzer's reagent; in catalysts. *Human Toxicity: See* Copper.

Cupric Nitrate. $Cu(NO_3)_2$; mol wt 187.56. Cu 33.88%, N 14.94%, O 51.18%. Prepn: *Gmelin's Handb. anorg. Chem.*, System No. 60 (Copper), part B, 8th ed, pp 164-179 (1958); Addison, Hathaway, *J. Chem. Soc.* 1958, 3099.

Large, blue-green, deliquesc, orthorhombic crystals. Sublimes at 150-225°. mp 255-256°. Soluble in water, ethyl acetate, dioxane; dissolves in and reacts vigorously with ether. *Keep well closed.*

Trihydrate, $Cu(NO_3)_2.3H_2O$, *gerhardite.* Blue, deliquesc, rhombic plates. mp 114.5°; d 2.05. Freely sol in water, alcohol; practically insol in ethyl acetate. pH of 0.2 molar aq soln 4.0. *Keep well closed.*

Hexahydrate, $Cu(NO_3)_2.6H_2O$, blue, deliquesc prismatic crystals. Loses $3H_2O$ at 26.4°. d 2.07. Freely sol in water; sol in alc. *Keep well closed.*

USE: In light-sensitive reproductive papers; as ceramic color; as mordant and oxidant in textile dyeing and printing; as reagent for burnishing iron, for giving a black "antique" finish to copper, for coloring zinc brown; in nickel-plating baths; in aluminum brighteners; in wood-preservatives, fungicides, herbicides; in pyrotechnic compositions; as catalyst component in solid rocket fuel; as nitrating agent for aromatic organosilicon compds; as catalyst for organic reactions. *Human Toxicity:* Irritating to skin, mucous membranes. *See also* Copper.

Cupric Oleate. $Cu(C_{18}H_{33}O_2)_2$; mol wt 626.43. $C_{36}H_{66}CuO_4$; C 69.02%, H 10.62%, Cu 10.14%, O 10.22%. Prepd from $CuSO_4$ and K oleate: Nelson, Pink, *J. Chem. Soc.* 1954, 4412.

Blue to green solid. Practically insol in water; slightly sol in alcohol; sol in ether.

USE: In antifouling compositions; as emulsifier and dispersing agent; as antioxidant in lubricating oils; as combustion-improver in fuel oils; as stabilizer for amide polymers; as catalyst. *Human Toxicity: See* Copper.

Cupric Oxalate. CuC_2O_4; mol wt 151.16. C 15.85%, Cu 41.92%, O 42.23%. Prepd by reaction of $CuSO_4$ with oxalic acid: David, *Bull. Soc. Chim. France* 1960, 719. Usually contains some water.

Blue-white powder. Loses any hydrated water by 200°; dec in air at 310° to CuO. Practically insol in water, alcohol, ether, acetic acid; sol in NH_4OH.

USE: As catalyst for organic reactions; as stabilizer for acetylated polyformaldehyde; in anticaries compositions; in seed treatments to repel birds and rodents.

Cupric Oxide. Black copper oxide. CuO; mol wt 79.54. Cu 79.88%, O 20.12%. Occurs in nature as the minerals *tenorite* (triclinic crystals) and *paramelaconite* (tetrahedral, cubic crystals). Prepn: Glemser, Sauer in *Handbook of Preparative Inorganic Chemistry*, G. Brauer, Ed., (2nd ed, Academic Press, 1965), p 1012.

Black to brownish-black amorphous or cryst powder or granules. d_4^{14} 6.315. Practically insol in water, alcohol; sol in dil acids, alkali cyanides, $(NH_4)_2CO_3$ soln; slowly sol in NH_3.

USE: As pigment in glass, ceramics, enamels, porcelain glazes, artificial gems; in manuf of rayon, other Cu compds; in sweetening petr gases; in galvanic electrodes; as flux in Cu metallurgy; in correcting Cu deficiencies in soil; as optical-glass polishing agent; to impart flux- and abrasion-resistance to glass fibers; in antifouling paints, pyrotechnic compositions; welding fluxes for bronze; as exciter in phosphor mixtures; as catalyst for organic reactions. *Human Toxicity: See* Copper.

Cupric Perchlorate. $Cu(ClO_4)_2$; mol wt 262.45. Cl 27.02%, Cu 24.21%, O 48.77%. Prepd from $Cu(NO_3)_2$ and perchloric acid or nitrosyl perchlorate: Caven, Bryce, *J. Chem. Soc.* 1934, 514; Hathaway, Underhill, *ibid.* 1960, 648; 1961, 3091; Hathaway, *Proc. Chem. Soc.* 1958, 344.

Very pale green, hygroscopic crystals. Volatilize on heating. Thermally stable to 130°; above 130° dec to a basic perchlorate. mp about 230-240°. Soluble in water, ether, dioxane, ethyl acetate. Practically insol in benzene, CCl_4, hexane.

Hexahydrate, $Cu(ClO_4)_2.6H_2O$, deep blue, monoclinic crystals. Freely sol in water, methanol, ethanol, acetic acid, acetic anhydride, acetone; slightly sol in ether, ethyl acetate.

USE: Analytical reagent: Kolb, *Ind. Eng. Chem., Anal. Ed.* 11, 197 (1939); 16, 38 (1944); Serjeant, *Nature* 186, 963 (1960). Also in copper electrodeposition; in catalysts for combustion and propellants.

Cupric *p*-Phenolsulfonate. *p-Hydroxybenzenesulfonic acid copper salt;* cupric sulfocarbolate; Cupriaseptol. $Cu[C_6H_4(OH)SO_3]_2$; mol wt 409.86. $C_{12}H_{10}CuO_8S_2$; C

Citrates

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GROUP 3: SEQUESTRANTS

(Chelating Agents, Metal Scavengers, Emulsifier Salts, Texturizers, Stabilizing Agents)

Name	Function, Usage	Levels of Use
Acetate, Calcium Acetate, Calcium di- Acetate, Potassium Acetate, Sodium di-	Beverages Baked goods	0.01 - 0.02% 0.02 - 0.05%
Calcium Salts Calcium Acetate Calcium Chloride Calcium Citrate Calcium Diacetate Calcium Gluconate Calcium Phosphate, monobasic Calcium Phytate Calcium Sulfate	Emulsifier salts Evaporated milk. Calcium chloride, up to Frozen desserts. Calcium sulfate	 0.1%
Citrate, Isopropyl (Monoisopropyl citrate)	Sequestrant, antioxidant Oleomargarine, salad oil. Up to General food use. Up to Animal fats and shortenings. Up to	 0.02% 0.02% 0.01%
Citrate, Monoglyceride	Synergist and solubilizer for antioxidant formulations for oils and fats. Up to	0.02%
Citrate, Stearyl	Metal scavenger, antioxidant. Up to Oleomargarine. Up to	0.15% 0.15%
Citrate, Triethyl	Dried egg whites. Up to	0.25%
Citrate Salts Calcium Citrate Potassium Citrate	Plasticizers for cheese spread, emulsifier salts Pasteurized process cheeses and cheese foods. Up to	 3.0%
Sodium Citrate	Cream (prevents "cream plug") Cream (prevents "feathering" in coffee cream) Ice cream emulsifier Processed cheese. Up to Evaporated milk. Up to Various cheeses	0.1% 0.012 - 0.37% 0.04% 3.0% 0.1%
Citric Acid	Lard Frozen peaches Grape wine Canned fish cakes Pie-crust mix Prepared breakfast cereal Soup base Antioxidant salt Used to assist dispersion of finings in brewing industry. Up to Oleomargarine Rendered animal fat or a combination of such fat and vegetable fat. Up to	0.001 - 0.01% 0.05% 0.05% 0.002% 0.0002% 0.035% 0.005% 0.01%
Ethylenediamine Tetraacetate, (EDTA) Calcium Disodium Salt of EDTA Disodium Dihydrogen Salt of EDTA	Carbonated beverages Crabmeat (cooked canned), retard struvite formation, promote color retention Dressings, nonstandardized	.0035% .0275% .0075%

EDTA

Name	Function, Usage	Levels of Use
Ethylenediamine Tetraacetate, (EDTA) (cont'd)	Fermented malt beverages	.0025%
	Salad dressing, french dressing, mayonnaise, sauces	.0075%
	Oleomargarine	.0075%
	Pecan-pie filling	.01%
	Potato salad	.01%
	Sandwich spread	.01%
	Shrimp (cooked canned), retard struvite formation, promote color retention	.025%
	Spice extractives in soluble carriers	.006%
	Processed dry pinto beans, promote color retention	.08%
	Canned carbonated soft drinks	.0035%
	Aqueous multivitamin preparations	.015%
	Vinegar	.02%
	Clams (cooked canned), promote color retention	.034%
	With Calcium Disodium, EDTA	
	Dressings, nonstandardized	.0075%
	Sauces	.0075%
	Sandwich spread	.01%
	Canned kidney beans	.0165%
✓ Gluconate, Calcium Gluconate, Sodium Oxystearin (gluconates)	Sequestrants	
	Used in cottonseed and soybean cooking and salad oils. Up to	.125%
	Dressings for foods	
Phosphates	Emulsifier salts, texturizers, sequestrants	
✓ Monocalcium Acid Phosphate Potassium Phosphate, dibasic Sodium Phosphate, dibasic (Disodium orthophosphate) Sodium Phosphate, monobasic (Monosodium orthophosphate)	Evaporated milk. Disodium phosphate or sodium citrate or both, or calcium chloride, added in a total quantity up to 0.1% by weight of the finished evaporated milk	
Sodium Phosphate, tribasic (Trisodium orthophosphate)	Pasteurized process cheeses, cheese spreads, and cheese foods. Up to	3.0%
	Ice cream. Disodium phosphate used to prevent thickening of chocolate sirup, up to	0.2%
Phosphate, Calcium hexameta- (Calcium metaphosphate) Phosphate, Sodium hexameta- (Sodium metaphosphate)	Emulsifiers, sequestering agents, texturizers	
	Breakfast cereals	0.27 - 0.3%
	Angel food cake. Up to	1.0%
	Flaked fish (prevents struvite formation)	0.5%
	Ice cream, ice milk	0.05%
	Bottled beverages, reconstituted lemon juice	0.02 - 0.7%
	Puddings	
	Processed cheeses	
	Artificially sweetened jellies, preserves. Sodium hexametaphosphate, up to	0.5%
	Potable water supplies in order to prevent scale formation and corrosion, up to	10 ppm
	Pumping pickle for curing hams, shoulders, etc. The finished product may contain up to	0.5%
Phosphate, Sodium Aluminum	Various cheeses	
Phosphate, Sodium pyro- (Sodium tetrapyrophosphate) (Tetrasodium pyrophosphate) (Sodium acid pyrophosphate)	Emulsifier salt, texturizer	
	Cold-water puddings. Up to	2.0%
	Processed cheeses. See phosphates	

Name	Function, Usage	Levels of Use
Ammonium Sulfate	Buffer	
	Bakery Products. See use for Ammonium Phosphate	
Calcium Carbonate	Alkali	
	Baking powder, up to	50%
	To reduce excessive acidity in wine	
	Neutralizer for ice cream and ice cream sirups	
	Confections	2.5%
Calcium Chloride	Confections	0.25%
Calcium Gluconate	Buffer	
	Confections	0.25%
Calcium Hydroxide (Calcium hydrate)	Alkali	
	Calcium sucrate, or saccharate, made up of three parts of sugar to one part of calcium hydroxide, is used to standardize acidity of frozen dairy products	
	Used to stabilize the potassium iodide of iodized salt	0.1%
	To reduce excessive acidity in wine	
	Sour-cream butter neutralizer	
	Canned peas. In such quantity that the pH does not exceed 8.0	
Calcium Lactate	Buffer	
	Constituent of some baking powders	
	Confections	0.25%
Calcium Oxide (Lime)	Alkali	
	Neutralizer in dairy industry (ice-cream mixes)	
	Sour-cream butter	
	Confections	
	In manufacture of tripe, sufficient for purpose	0.25%
Calcium Sulfate	Creamed cottage cheese	
Carbonate, Potassium Carbonate, Potassium bi-	Alkali	
	Used in combination with potassium hydroxide in extraction of color from annatto	
	Confections	
	Cacao products. Same as for Ammonium Carbonate	3.0%
Carbonate, Sodium Carbonate, Sodium sesqui-	Alkali	
Carbonates	Neutralizer for butter, cream, fluid milk, ice cream	
	Processing of olives before canning	
	Cacao products. Same as for Ammonium Carbonate	
	Canned peas. Same as for Magnesium Carbonate	
Carbonate, Sodium bi-	Alkali	
	Prepared pancake, biscuit, muffin mixes	
	Leavening agent in baking powders	
	Various crackers and cookies	
	Tomato soup (adjust acidity)	
	Neutralizer for ices and sherbets; sirups for frozen products	
	Sour-cream butter	
	Confections	
	Cacao products. Same as for Ammonium Carbonate	1.0%
	Self-rising flours, self-rising white and yellow cornmeals. Combined weight of acid-reacting substances (monocalcium	

Name	Function, Usage	Levels of Use
Cottonseed Flour, cooked (partially defatted and toasted)		
Ferrous Gluconate		
FD&C Blue No. 1 (Brilliant blue) FD&C Blue No. 2 (Indigo carmine) FD&C Green No. 1 (Guinea green B) FD&C Green No. 2 (Light green S F yellowish) FD&C Green No. 3 (Fast green FCF) FD&C Red No. 2 (Amaranth)	Bottled soft drinks	
	Mint-flavored jelly	0.00013%
	Breakfast cereals	0.0004%
	Imitation jellies Bottled soft drinks	0.005 - 0.008%
FD&C Red No. 3 (Erythrosine) FD&C Violet No. 1 FD&C Yellow No. 5 (Tartrazine)	Canned fruit cocktail, fruit salad Cherry-pie mix	0.0056% 0.01%
	Prepared breakfast cereal	0.004%
	Imitation strawberry jelly	0.002%
	Bottled soft drinks	
FD&C Yellow No. 6 (Sunset yellow FCF)	Bottled soft drinks	
FD&C Lakes (Aluminum or calcium lakes of FD&C certified colors)	Used for dyeing shell eggs	
Grapeskin Extract		
Iron Oxides		
Paprika and Paprika Oleoresin		
Riboflavin		
Saffron	Vegetable dye prepared from American saffron (safflower)	
	Meat products	
Titanium Dioxide	Colorant	
	White pigment for candy. Up to Gums, marking ink for confectionery	0.4%
Tumeric and Curcumin	Vegetable dye	
	Meat products	
Xanthophyll		

Name	Function, Usage	Levels of Use
Copper Sources: Cupric Chloride Cupric Gluconate Cupric Sulfate Cupric Oxide	Mineral supplement Copper per day up to In any food, up to	 2 mg 0.005%
Cysteine (L- form)	Essential amino acid Bakery products. Per 100 lbs flour	 0.009 lbs
Cystine (L- and DL- forms)	Amino acid	
Fluorine Sources: Calcium Fluoride Hydrofluosilicic Acid Potassium Fluoride Sodium Fluoride Sodium Silicofluoride	Fluoridation of water	
Folic Acid	Nutrient Per day (except on prescription), up to	 0.10 mg
Histidine (L- and DL- forms)	Essential amino acid	
Inositol	Dietary supplement	
Iodine Sources:	Essential nutrient	
Iodine (from dehydrated kelp)	Iodine per day, up to	0.7 mg
Cuprous Iodide	Table salt, up to	0.01%
Potassium Iodate	Dietary supplement, iodine per day, up to	0.15 mg
Potassium Iodide	Table salt, up to Dietary supplement, iodine per day, up to	0.01% 0.15%
Iron Sources: Iron (Reduced iron, iron powder) Iron Salts Ferric Choline Citrate Ferric Phosphate Ferric Pyrophosphate Ferric Sodium Pyrophosphate (Sodium iron pyrophosphate) Ferrous Fumarate Ferrous Gluconate Ferrous Lactate Ferrous Sulfate	Mineral supplement Prepared breakfast cereal Poultry stuffing Per lb of enriched flour, enriched bromated flour, enriched self-rising flour, enriched macaroni and noodle products Enriched farina, per lb Per lb of enriched cornmeal, enriched corn grits Per lb of enriched bread, rolls, etc. Iron salts may be used for enriched products if harmless and assimilable	 13 - 16.5 mg 13 mg 13 - 26 mg 8 - 12.5 mg
Isoleucine (L- and DL- forms)	Essential amino acid	
Leucine (L- and DL- forms)	Essential amino acid	
Linoleic Acid (prepared from edible fats and oils and free from chick-edema factor)	Essential fatty acid	
Liver-stomach concentrate (with intrinsic factor complex)	Dietary supplement	
Lysine (L- and DL- forms) l-Lysine Monohydrochloride	Essential amino acids Fortification of specialty bread and cereal mixes, of weight of flour	 0.25 - 0.5%

Name	Function, Usage	Levels of Use
Magnesium Sources: Magnesium Oxide Magnesium Phosphate (dibasic and tribasic) Magnesium Sulfate	Mineral supplement	
Manganese Sources: Manganese Chloride Manganese Citrate Manganese Gluconate Manganese Glycerophosphate Manganese Hypophosphite Manganese Sulfate Manganese Oxide Manganous Oxide	Mineral supplement	
DL-Methionine	Essential amino acid	
	Per day, up to	200 mg
Molybdenum Sources: Ammonium Molybdate Sodium Molybdate Molybdenum Sesquioxide Molybdenum Trioxide	Mineral supplement	
	Per day, up to	2 mg
Niacin (Nicotinic Acid) Niacinamide (Nicotinic amide, Nicotinamide) Aluminum Nicotinate	Essential nutrient	
	Prepared breakfast cereal, peanut butter, baby cereals	0.002 - 0.005%
	Per lb of enriched flour, enriched bromated flour, enriched self-rising flour	16 - 20 mg
	Enriched farina. Per lb	16 - 20 mg
	Enriched cornmeal, enriched corn grits. Per lb	16 - 24 mg
	Enriched macaroni and noodle products. Per lb	27 - 34 mg
	Enriched bread, rolls, etc. Per lb	10 - 15 mg
Nickel Sulfate	Mineral supplement	
	Nickel per day, up to	1 mg
d-Pantothenamide	Source of pantothenic acid activity in foods for special dietary use	
Pantothenate, Calcium Pantothenate, Sodium d-Pantothenyl Alcohol	B-complex vitamin	
Phenylalanine (L- and DL-forms)	Essential amino acid	
Phosphorous Sources: Calcium Phosphate (monobasic, dibasic, and tribasic) Magnesium Phosphate (dibasic and tribasic) Potassium Glycerophosphate Sodium Phosphate (monobasic, dibasic, and tribasic)	Mineral supplements	
	Constituents of formulated mineral supplements for cereal products, particularly breakfast foods such as farina	
	Prepared cereals, up to	0.5%
Potassium Chloride	Substitute for sodium chloride in low-sodium dietary foods	
Proline (L- and DL- forms)	Amino acid supplement	
Pyridoxine Hydrochloride (Vitamin B_6)	B-complex vitamin	
	Evaporated milk-base foods for infants	

Name	Function, Usage	Levels of Use
Beeswax (Yellow wax, bleached (white) wax)	Candy glaze and polish. Up to	0.4%
Bentonite	Clarifying agent in wine, etc.	
Bromelin	Enzyme for tenderizing meats	
Brominated Vegetable Oils	Clouding agent. These high-density oils are blended with low-density essential oils to make them easier to emulsify	
	Used largely in soft drinks	0.0007 - 0.06%
	Citrus-flavored beverages	0.03 - 0.05%
	Ice cream, ices	0.001 - 0.06%
	Baked goods	0.0015 - 0.02%
Butadiene-Styrene Copolymer	Chewing-gum base component	
Butane	Gas	
Butyl Rubber, without inhibitor (chewing-gum grade)	Chewing-gum base component	
Caffeine	Stimulant	
	Cola beverages. Up to	0.02%
Calcium Carbonate	Yeast food, firming agent, carrier	
	Used in candies; in hard candies to prevent sticking; in fudge to promote creaming. Up to	2.5%
	Cereal flours. Carrier for bleaching ingredient. One part benzoyl peroxide per six parts carrier, maximum	
	Bread, rolls, buns, etc. See Ammonium Chloride	
Calcium Chloride	Firming agent	
	Used to firm sliced apples and other fruit	0.05%
	Apple-pie mix, for firming slices	0.03%
	Jelling ingredient	
	Certain cheeses. Up to 0.02% of the weight of the milk is added as an optional ingredient to aid coagulation	
	Artificially sweetened fruit jelly in amount necessary	
	Canned potatoes. Calcium content of the finished product, up to	0.051%
	Canned tomatoes. Calcium content of the finished product, up to	0.026%
Calcium Citrate	Firming agent	
	Jelling ingredient	
	Artificially sweetened fruit jelly in amount necessary	
	Canned potatoes, canned sweet peppers. Calcium content of the finished product, up to	0.51%
	Canned tomatoes. Calcium content of the finished product, up to	0.026%
Calcium Gluconate	Firming agent	
	Firming tomatoes, apple slices	
Calcium Hydroxide	Firming agent	
	Firming various fruit products	

Name	Function, Usage	Levels of Use
Calcium Lactate Calcium Oxide	Yeast food, dough conditioners	
	Bread, rolls, buns, etc. Same as for Ammonium Chloride	
Calcium Phosphate, dibasic (Dicalcium orthophosphate)	Yeast food, dough conditioner	
	Constituent of bread improvers per 100 lbs of flour	0.25 lbs
	Cereal flours. Carrier for bleaching ingredient. One part benzoyl peroxide per six parts of carrier, maximum	
	Bread, rolls, buns, etc. See Ammonium Chloride	
Calcium Phosphate, monobasic (Monocalcium orthophosphate, monocalcium acid phosphate)	Yeast food, dough conditioner, firming agent	
	Jelling ingredient	
	Bread, rolls, buns, etc.	
	Artificially sweetened fruit jelly	
	For canned potatoes, canned sweet peppers, and canned tomatoes, see Calcium Citrate	
Calcium Phosphate, tribasic	Anti-caking agent	
	Table salt	1.0%
	Powdered sugar	1.5%
	Malted milk powder	1.0%
	Condiments	0.0047 - 0.054%
	Puddings	0.05 - 0.1%
	Meat dry-curing mixtures	
	Cereal flours. See Calcium Phosphate, dibasic	
	Vanilla powder. Anti-caking ingredient. Total weight of such ingredients up to	2.0%
Calcium Salts	Firming agent	
	Potatoes. Purified calcium chloride, calcium citrate, calcium sulfate, monocalcium phosphate, or any mixture of two or more such calcium salts in a quantity reasonably necessary to firm the potatoes, up to	0.051%
	Canned tomatoes. Purified calcium salts as for potatoes, up to	0.026%
Calcium Salts of fatty acids	Binder, anti-caking agent	
Calcium Sulfate	Yeast food, dough conditioner, firming agent	
	Yeast food in brewing and other fermentation industries	
	Production of Spanish type or flor sherry, as potassium sulfate, up to	0.2%
	Jelling ingredient	
	Cereal flours. Carrier for bleaching agent. One part benzoyl peroxide per six parts carrier, maximum	
	Bread, rolls, buns, etc. See Ammonium Chloride	
	Blue cheese	
	Gorgonzola cheese. Bleach ingredient	
	Artificially sweetened fruit jelly	
	For canned potatoes, canned sweet peppers, and canned tomatoes. See Calcium Citrate	
Carbon Dioxide	Pressure-dispensing agent	
	Gassed creams (pressure-dispensed whipped cream)	
Casein Ammonium Caseinate Calcium Caseinate Potassium Caseinate Sodium Caseinate	Texturizer	
	Ice cream, frozen custard, ice milk, fruit sherbets	

Name	Function, Usage	Levels of Use
Ethylenediamine Tetraacetate, (EDTA) (cont'd)	Fermented malt beverages	.0025%
	Salad dressing, french dressing, mayonnaise, sauces	.0075%
	Oleomargarine	.0075%
	Pecan-pie filling	.01%
	Potato salad	.01%
	Sandwich spread	.01%
	Shrimp (cooked canned), retard struvite formation, promote color retention	.025%
	Spice extractives in soluble carriers	.006%
	Processed dry pinto beans, promote color retention	.08%
	Canned carbonated soft drinks	.0035%
	Aqueous multivitamin preparations	.015%
	Vinegar	.02%
	Clams (cooked canned), promote color retention	.034%
	With Calcium Disodium, EDTA	
	Dressings, nonstandardized	.0075%
	Sauces	.0075%
	Sandwich spread	.01%
	Canned kidney beans	.0165%
Gluconate, Calcium Gluconate, Sodium	Sequestrants	
Oxystearin	Used in cottonseed and soybean cooking and salad oils. Up to	.125%
	Dressings for foods	
Phosphates	Emulsifier salts, texturizers, sequestrants	
Monocalcium Acid Phosphate Potassium Phosphate, dibasic Sodium Phosphate, dibasic (Disodium orthophosphate) Sodium Phosphate, monobasic (Monosodium orthophosphate)	Evaporated milk. Disodium phosphate or sodium citrate or both, or calcium chloride, added in a total quantity up to 0.1% by weight of the finished evaporated milk	
Sodium Phosphate, tribasic (Trisodium orthophosphate)	Pasteurized process cheeses, cheese spreads, and cheese foods. Up to	3.0%
	Ice cream. Disodium phosphate used to prevent thickening of chocolate sirup, up to	0.2%
Phosphate, Calcium hexameta- (Calcium metaphosphate)	Emulsifiers, sequestering agents, texturizers	
Phosphate, Sodium hexameta- (Sodium metaphosphate)	Breakfast cereals	0.27 - 0.3%
	Angel food cake. Up to	1.0%
	Flaked fish (prevents struvite formation)	0.5%
	Ice cream, ice milk	0.05%
	Bottled beverages, reconstituted lemon juice Puddings Processed cheeses	0.02 - 0.7%
	Artificially sweetened jellies, preserves. Sodium hexametaphosphate, up to	0.5%
	Potable water supplies in order to prevent scale formation and corrosion, up to	10 ppm
	Pumping pickle for curing hams, shoulders, etc. The finished product may contain up to	0.5%
Phosphate, Sodium Aluminum	Various cheeses	
Phosphate, Sodium pyro- (Sodium tetrapyrophosphate) (Tetrasodium pyrophosphate) (Sodium acid pyrophosphate)	Emulsifier salt, texturizer	
	Cold-water puddings. Up to	2.0%
	Processed cheeses. See phosphates	

Name	Function, Usage	Levels of Use
Ammonium Sulfate	Buffer	
	Bakery Products. See use for Ammonium Phosphate	
Calcium Carbonate	Alkali	
	Baking powder, up to	50%
	To reduce excessive acidity in wine	
	Neutralizer for ice cream and ice cream sirups	
	Confections	2.5%
Calcium Chloride	Confections	0.25%
Calcium Gluconate	Buffer	
	Confections	0.25%
Calcium Hydroxide (Calcium hydrate)	Alkali	
	Calcium sucrate, or saccharate, made up of three parts of sugar to one part of calcium hydroxide, is used to standardize acidity of frozen dairy products	
	Used to stabilize the potassium iodide of iodized salt	0.1%
	To reduce excessive acidity in wine	
	Sour-cream butter neutralizer	
	Canned peas. In such quantity that the pH does not exceed 8.0	
Calcium Lactate	Buffer	
	Constituent of some baking powders	
	Confections	0.25%
Calcium Oxide (Lime)	Alkali	
	Neutralizer in dairy industry (ice-cream mixes)	
	Sour-cream butter	
	Confections	0.25%
	In manufacture of tripe, sufficient for purpose	
Calcium Sulfate	Creamed cottage cheese	
Carbonate, Potassium Carbonate, Potassium bi-	Alkali	
	Used in combination with potassium hydroxide in extraction of color from annatto	
	Confections	3.0%
	Cacao products. Same as for Ammonium Carbonate	
Carbonate, Sodium Carbonate, Sodium sesqui-	Alkali	
	Neutralizer for butter, cream, fluid milk, ice cream	
	Processing of olives before canning	
	Cacao products. Same as for Ammonium Carbonate	
	Canned peas. Same as for Magnesium Carbonate	
Carbonate, Sodium bi-	Alkali	
	Prepared pancake, biscuit, muffin mixes	
	Leavening agent in baking powders	
	Various crackers and cookies	
	Tomato soup (adjust acidity)	
	Neutralizer for ices and sherbets; sirups for frozen products	
	Sour-cream butter	
	Confections	1.0%
	Cacao products. Same as for Ammonium Carbonate	
	Self-rising flours, self-rising white and yellow cornmeals. Combined weight of acid-reacting substances (monocalcium	

Name	Function, Usage	Levels of Use
Carbonate, Sodium bi- (cont'd)	and	
	and	
	Cann	
	Tom	
	ton	
	Buffers	
Citrate, Calcium Citrate, Sodium	Con	
	Jell	
	ta	
	sa	
Citric Acid	Acid	
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Fumaric Acid	Ac	
Glucono-delta-Lactone	Ac	
Hydrochloric Acid	A	

Name	Function, Usage	Levels of Use
Beeswax (Yellow wax, bleached (white) wax)	Candy glaze and polish. Up to	0.4%
Bentonite	Clarifying agent in wine, etc.	
Bromelin	Enzyme for tenderizing meats	
Brominated Vegetable Oils	Clouding agent. These high-density oils are blended with low-density essential oils to make them easier to emulsify	
	Used largely in soft drinks	0.0007 - 0.06%
	Citrus-flavored beverages	0.03 - 0.05%
	Ice cream, ices	0.001 - 0.06%
	Baked goods	0.0015 - 0.02%
Butadiene-Styrene Copolymer	Chewing-gum base component	
Butane	Gas	
Butyl Rubber, without inhibitor (chewing-gum grade)	Chewing-gum base component	
Caffeine	Stimulant	
	Cola beverages. Up to	0.02%
Calcium Carbonate	Yeast food, firming agent, carrier	
	Used in candies; in hard candies to prevent sticking; in fudge to promote creaming. Up to	2.5%
	Cereal flours. Carrier for bleaching ingredient. One part benzoyl peroxide per six parts carrier, maximum	
	Bread, rolls, buns, etc. See Ammonium Chloride	
Calcium Chloride	Firming agent	
	Used to firm sliced apples and other fruit	0.05%
	Apple-pie mix, for firming slices	0.03%
	Jelling ingredient	
	Certain cheeses. Up to 0.02% of the weight of the milk is added as an optional ingredient to aid coagulation	
	Artificially sweetened fruit jelly in amount necessary	
	Canned potatoes. Calcium content of the finished product, up to	0.051%
	Canned tomatoes. Calcium content of the finished product, up to	0.026%
Calcium Citrate	Firming agent	
	Jelling ingredient	
	Artificially sweetened fruit jelly in amount necessary	
	Canned potatoes, canned sweet peppers. Calcium content of the finished product, up to	0.51%
	Canned tomatoes. Calcium content of the finished product, up to	0.026%
Calcium Gluconate	Firming agent	
	Firming tomatoes, apple slices	
Calcium Hydroxide	Firming agent	
	Firming various fruit products	

Name	Function, Usage	
Copper Sources:	Mineral supplement	
Cupric Chloride	Copper per day up to	
Cupric Gluconate	In any food, up to	
Cupric Sulfate		
Cupric Oxide		
Cysteine (L- form)	Essential amino acid	
	Bakery products. Per 100 lbs flour	
Cystine (L- and DL- forms)	Amino acid	
Fluorine Sources:	Fluoridation of water	
Calcium Fluoride		
Hydrofluosilicic Acid		
Potassium Fluoride		
Sodium Fluoride		
Sodium Silicofluoride		
Folic Acid	Nutrient	
	Per day (except on prescription), up to	
Histidine (L- and DL- forms)	Essential amino acid	
Inositol	Dietary supplement	
Iodine Sources:	Essential nutrient	
	Iodine per day, up to	
Iodine (from dehydrated kelp)	Table salt, up to	
Cuprous Iodide	Dietary supplement, iodine per day, up to	
Potassium Iodate	Table salt, up to	
Potassium Iodide	Dietary supplement, iodine per day, up to	
Iron Sources:	Mineral supplement	
Iron (Reduced iron, iron powder)	Prepared breakfast cereal	
Iron Salts	Poultry stuffing	
Ferric Choline Citrate	Per lb of enriched flour, enriched bromated flour, enriched self-rising flour, enriched macaroni and noodle products	
Ferric Phosphate		
Ferric Pyrophosphate	Enriched farina, per lb	
Ferric Sodium Pyrophosphate (Sodium iron pyrophosphate)	Per lb of enriched cornmeal, enriched corn grits	
Ferrous Fumarate	Per lb of enriched bread, rolls, etc.	
Ferrous Gluconate	Iron salts may be used for enriched products if harmless and assimilable	
Ferrous Lactate		
Ferrous Sulfate		
Isoleucine (L- and DL- forms)	Essential amino acid	
Leucine (L- and DL- forms)	Essential amino acid	
Linoleic Acid (prepared from edible fats and oils and free from chick-edema factor)	Essential fatty acid	
Liver-stomach concentrate (with intrinsic factor complex)	Dietary supplement	
Lysine (L- and DL- forms) 1-Lysine Monohydrochloride	Essential amino acids	
	Fortification of specialty bread and cereal mixes, of weight of flour	0.25 - 0.5%

	Function, Usage	Levels of Use
	Mineral supplement	
	Mineral supplement	
	Essential amino acid	
	Per day, up to	200 mg
	Mineral supplement	
	Per day, up to	2 mg
	Essential nutrient	
	Prepared breakfast cereal, peanut butter, baby cereals	0.002 - 0.005%
	Per lb of enriched flour, enriched bromated flour, enriched self-rising flour	16 - 20 mg
	Enriched farina. Per lb	16 - 20 mg
	Enriched cornmeal, enriched corn grits. Per lb	16 - 24 mg
	Enriched macaroni and noodle products. Per lb	27 - 34 mg
	Enriched bread, rolls, etc. Per lb	10 - 15 mg
	Mineral supplement	
	Nickel per day, up to	1 mg
	Source of pantothenic acid activity in foods for special dietary use	
	B-complex vitamin	
	Essential amino acid	
	Mineral supplements	
	Constituents of formulated mineral supplements for cereal products, particularly breakfast foods such as farina	
	Prepared cereals, up to	0.5%
	Substitute for sodium chloride in low-sodium dietary foods	
	Amino acid supplement	
	B-complex vitamin	
	Evaporated milk-base foods for infants	

Name	Function, Usage	Levels of Use
...st, Dried ...st, Dried, Irradiated ...st, Torula, Dried	Dietary source of folic acid	
	Folic acid per gram of yeast	.04 mg
	Pteroylglutamic acid per gram of yeast	.008 mg
	Enriched farina. Irradiated yeast may be added as source of vitamin D	
	Enriched cornmeals and corn grits	
	Bakery products	
Zinc Sources: Zinc Chloride Zinc Gluconate Zinc Oxide Zinc Stearate (prepared from stearic acid free from chick-edema factor) Zinc Sulfate	Mineral supplement	

COMPREHENSIVE GRAS SURVEY -- NAS/ARC 1972

-- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY.
BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - -

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
CALCIUM GLUCONATE NAS 0041	01	BAKED GOODS(R)	*	0-5 MO.	54.106240	71.611200	54.106240
				6-11 MO.	402.208440	824.324480	402.208440
				12-23 MO.	667.291200	1429.041200	667.291200
				2-65+ YR.	2183.345920	3243.191680	2183.345920
CALCIUM GLUCONATE NAS 0041	20	GELATIN PUD(R)	*	0-5 MO.	46.886000	63.296100	61.656600
				6-11 MO.	300.670400	909.584400	394.688240
				12-23 MO.	323.313400	787.684800	423.[illegible]240
				2-65+ YR.	478.237200	1230.757500	628.297320
CALCIUM GLUCONATE NAS 0041	28	IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	5.600000	9.200000	5.600000
				12-23 MO.	3.200000	13.600000	3.200000
				2-65+ YR.	3.600000	6.000000	3.600000
CALCIUM GLUCONATE NAS 0041	33	SUGAR SUBS(R)	*	0-5 MO.	**********	**********	**********
				6-11 MO.	.000900	.001800	.000900
				12-23 MO.	**********	.001800	**********
				2-65+ YR.	.007200	.007200	.007200
CALCIUM GLUCONATE NAS 0041	ALL CATEGORIES ********** ********** **********		7	0-5 MO.	100.992240	134.907300	115.762840
				6-11 MO.	709.879740	1743.114680	804.408580
				12-23 MO.	1154.004600	2230.327880	1295.921740
				2-65+ YR.	2665.190320	4479.956380	2815.850440
COPPER GLUCONATE NAS 0060	05	MILK PROD(R)	*	0-5 MO.	.081000	.000000	.270000
				6-11 MO.	.936000	4.501500	3.120000
				12-23 MO.	.817500	2.610000	2.725000
				2-65+ YR.	.592500	1.800000	1.975000

-- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II). PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE --

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
COPPER GLUCONATE NAS 0069	06 PROCSD FRUT(R)	*	0-5 MO.	3.496800	9.374400	3.525000
			6-11 MO.	38.539200	99.976000	38.850000
			12-23 MO.	74.644400	148.576800	75.450000
			2-65+ YR.	88.015200	186.446400	88.725000
COPPER GLUCONATE NAS 0069	16 SOFT CANDY(R)	*	0-5 MO.	.009000	.090000	.010000
			6-11 MO.	.099000	.306000	.110000
			12-23 MO.	.157500	.418500	.176000
			2-65+ YR.	.261000	.792000	.290000
COPPER GLUCONATE NAS 0069	22 SNACK FOODS(R)	*	0-5 MO.	**********	.000000	**********
			6-11 MO.	.000000	.000000	.000000
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	.000000	.000000	.000000
COPPER GLUCONATE NAS 0069	31 CHEWING GUM(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	.004710	.004710	.004710
			12-23 MO.	.004710	.014130	.004710
			2-65+ YR.	.009420	.018840	.009420
COPPER GLUCONATE NAS 0069	83 FORMULAS(B)	*	0-5 MO.	1.275660	2.337000	2.635800
			6-11 MO.	.259920	1.238420	.547200
			12-23 MO.	.003600	.023560	.176000
COPPER GLUCONATE NAS 0069	ALL CATEGORIES ********** ********** **********	7	0-5 MO.	4.852460	11.861400	6.490800
			6-11 MO.	39.636830	102.026630	42.631910
			12-23 MO.	75.905710	151.040900	78.530710
			2-65+ YR.	88.876120	189.066240	90.999420

-- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - -

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
FERROUS GLUCONATE NAS 0083	15 CCNDM RELSH(R)	*	0-5 MO.	**********	.002000	**********
			6-11 MO.	.016000	.044000	.016000
			12-23 MO.	.056000	.152000	.056000
			2-65+ YR.	.176000	.424000	.176000
FERROUS GLUCONATE NAS 0083	ALL CATEGORIES **********	*	0-5 MO.	**********	.002000	**********
			6-11 MO.	.016000	.044000	.016000
			12-23 MO.	.056000	.152000	.056000
			2-65+ YR.	.176000	.424000	.176000
SODIUM GLUCONATE NAS 0191	08 PROCSD FRUT(R)	*	0-5 MO.	7.990000	21.420000	7.990000
			6-11 MO.	88.000000	219.300000	88.000000
			12-23 MO.	171.020000	339.490000	171.020000
			2-65+ YR.	201.110000	426.020000	201.110000
SODIUM GLUCONATE NAS 0191	23 BEV TYPE I(R)	*	0-5 MO.	1.596480	2.394720	1.596480
			6-11 MO.	15.100040	51.606040	15.100040
			12-23 MO.	36.053840	108.095000	36.053840
			2-65+ YR.	69.180800	184.726040	69.180800
SODIUM GLUCONATE NAS 0191	ALL CATEGORIES **********	*	0-5 MO.	9.586480	23.814720	9.586480
			6-11 MO.	103.100040	270.906040	103.100040
			12-23 MO.	207.073840	447.585000	207.073840
			2-65+ YR.	270.290800	610.746040	270.290800

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/03/72

-- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
CALCIUM GLUCONATE NAS 6041	5/ 7	51,000	309,685	315,186			315,186
COPPER GLUCONATE NAS 6062	7/ 7	500	2,865	2,865			2,865
FERROUS GLUCONATE NAS 6083	*/ *	4,000	2,200	2,200			2,200
SODIUM GLUCONATE NAS 8151	*/ 4	0	40,300	49,495			49,495

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 — 10/01/72

-- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CALCIUM GLUCONATE NAS 0041	01	BAKED GOODS(R)	*	1.59136	1.59136
	20	GELATIN PUD(R)	*	2.34430	3.08283
	28	IMIT DAIRY(R)	*	.40000	.40000
	33	SUGAR SUBS(R)	*	.00900	.00900
COPPER GLUCONATE NAS 0069	05	MILK PRODS(R)	*	.00150	.00500
	08	PROCSD FRUT(R)	*	.07440	.07500
	16	SOFT CANDY(R)	*	.00450	.00600
	22	SNACK FOODS(R)	*	.00000	.00000
	31	CHEWING GUM(R)	*	.00471	.00471
FERROUS GLUCONATE NAS 0083	15	CONDR RELSH(R)	*	.00200	.00200
SODIUM GLUCONATE NAS 0191	08	PROCSD FRUT(R)	*	.17000	.17000
	23	BEV TYPE I(R)	*	.06652	.06652